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INNERVATION OF THE HYPOPHYSIS

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Certain lines of investigation on the physiology of the hypophysis indicate that a better knowledge of the nerve supply to this important organ is urgent. Most of our information on this subject has naturally been gleaned from work done on the common laboratory mammals. Due to species differences there is danger in generalizing too freely from observations made on a particular animal. Hence data obtained directly from man is of special importance in human physiology and clinical medicine.

The Hypophysis and the Hypothalamus

The connections of the hypophysis with the hypothalamus can no longer be questioned. This subject is admirably summarized by Fisher, Ingram and Ranson (1). Roussy and Mosinger (2) have conceived several elaborate neural circuits involving the hypophysis. The literature on the nuclear configuration of the hypothalamus is listed by Crouch (3) whereas the morphology, including the fiber connection, of this region of the human brain is presented in considerable detail by Greving (4). The number of recognizable nuclei in the hypothalamus is greatly reduced in man as compared with animals (5). For further details on the comparative anatomy, the reader is referred to Kappers, Huber and Crosby (6).

It is generally agreed that the supraoptic nuclei (fig. 1), and possibly to some extent the paraventricular (filiform) nuclei, are the sources of the tractus supraopticohypophyseus, which converges in the tuber cinereum to the infundibulum through which it enters the enlarged division of the neural lobe (processus infundibuli). Here it forms an exceedingly complicated plexus of nonmyelinated nerve fibers, which ramify to all parts of this lobe. Hypophysectomy results in degeneration of most, if not all, of the cells whose processes have thus been severed. In the rat nearly all the cells of the supraoptic nuclei have disappeared by 6 months after hypophysectomy (7). In the cat and some other animals, including the monkey (8), a smaller bundle of nerve fibers descends in the dorsal wall of the infundibulum. This apparently arises from cells located more posteriorly in the tuber cinereum, and is known as the tractus tuberohypophyseus. The two bundles together are designated the tractus hypothalamicohypophyseus. The infundibulum is largely occupied by these nerve fibers.

One is at a loss to reconcile the statement of Mahoney and Sheehan (9) that the sparseness of nerve fibers passing along the infundibular stem of the monkey, does not suggest any striking neural connection between the hypophysis and the hypothalamus. In a macaque at our disposal there are at least 40,000 fibers in the stem of the hypophysis. Because of the small size and arrangement of the fibers, this is probably an under-estimate rather than an over-estimate. For comparison it may be noted that this is more than the number of fibers in the vagus trunk of the cat.

Innervation of Pars Intermedia

Many investigators report that some nerve fibers pass from the infundibular process into pars intermedia. Ranson (10 and 10a) and Fisher (11) state that the number of such fibers in the rat, cat, and monkey is very small, and that they do not penetrate very far into pars intermedia. Hair (12) on the other hand, conveys the impression that they are plentiful in the cat, thus agreeing with earlier work on several other species, including man, by Cajal (13), Berkley (14), Gentes (15), Gemelli (16), Trautmann (17), Pines (18) and Roussy and Mosinger (19). Ibanez (20), also a recent worker in this field, found distinct nerve fibers in pars intermedia by the use of the Golgi method; but the relative number is not clear. Bodian (21), by a new silver technic, utilizing activated protargol, has clearly demonstrated fairly abundant nerve fibers in limited regions of this lobe in the cat and the monkey (macaque), but definite nerve endings about epithelial cells are rare (personal communication). Possibly the most widely copied illustration of the innervation of pars intermedia is one published in 1912 by Tello (22) and based on human material. His figure 9 shows numerous nerve fibers, not only between the epithelial cells forming the walls of the vesicles, but also ending intracellularly as a cup-shaped expansion against the nucleus. Terminals of this exact form have not been well confirmed by subsequent investigators. Hoening (23) merely states that in man the fibers terminate near the nucleus, and submits no illustrations. Berkeley (14) describes only simple endings. Ibanez (20), working in the laboratory with which Tello is associated, but using cats and dogs, shows only very simple intercellular terminals in pars intermedia. This is also true of Hair's (12) report. Bucy (24) describes a very large rounded knob on the end of an occasional nerve fiber in pars intermedia of cattle; but one gains the impression that these are incidental structures, if neural at all. Nerve fibers may appear to end in a so-called Herring body, which darkens with silver and thus give the appearance of enlarged nerve terminals. Serial sections show that in most of these instances the nerve fiber actually continues beyond the enlargement, and is merely passing through, or is only in contact with the amorphous mass. The great majority of these bodies are not related in any way to nerve fibers.

A number of investigators have thought that certain elongated cells in pars intermedia were of a sensory character. This is so improbable in higher animals that a discussion of this point does not seem profitable.

Connections of the Hypophysis with the Carotid Plexus

The prevailing view for a considerable period was that the hypophysis was principally innervated by fibers from the internal carotid plexus. This was based on gross dissections with the attendant difficulty of discriminating between connective tissue and nerve fibers, as pointed out by Henle (25). While Dandy (26), with a more specific technic applied to the dog, clearly followed many small nerve bundles from the carotid plexus along the hypophyseal vessels to the hypophysis

where the great majority become related to the anterior lobe (pars distalis), great difficulty has been encountered in attempting to demonstrate nerve fibers within this lobe. Gentes (15), Herring (27), Greving (28) and Bucy (24) could find none. Berkley (14), Thoan (29), Massay (30), Tello (22), Hoening (23) and Croll (31) report an occasional fiber or a few fibers in a very limited (usually peripheral) region only. Blair Bell (32) has published the only illustration that we could find of such fibers in pars distalis of the human hypophysis, but the description is not sufficient to indicate how extensive such nerves are. In the small area shown, many of the cells are completely surrounded by a nerve net arising apparently from a few profusely branched fibers. The cut is from a retouched photomicrograph of a Golgi preparation and is not convincing.

Pines (33) and Hair (12) contend that nerve fibers are plentiful in the anterior lobe. It is not clear, however, that this has been found throughout the entire lobe. Pines admits that he saw the nerve fibers usually at the periphery of the gland but occasionally also in the interior. It is likely that very limited areas have been taken as representative of the whole, on the assumption that large areas may show no nerve fibers because of faulty technic. The most recent contributors to this subject, Brooks and Gersh (34), claim to have found a terminal nerve net inclosing most of the surface of both acidophils and basophils of the hypophysis of the rat. This is hardly compatible with the scarcity of nerve fibers in the anterior lobe and needs further corroboration.

Experimental work on the origin of the nerve fibers of the anterior lobe is not wholly concordant. Maiman (35) reports that some of the cells of the superior cervical sympathetic ganglion were chromatolytic 12 to 16 days subsequent to destruction of the anterior lobe of the hypophysis of young dogs, while Mahoney (36) saw no changes in that ganglion 17 days after complete hypophysectomy in a chimpanzee. Incidentally, Gagel and Mahoney (37) could not be sure of any changes in the hypothalamic nuclei as a direct result of either hypophysectomy or severing the stalk in cats, dogs and monkeys. That some of the fibers in the anterior lobe come from cells not located in the cervical sympathetic ganglia is indicated by the work of Hair (12) and of Books and Gersh (34), for they noted that some fibers still remained in that lobe after bilateral cervical sympathectomy. Attempts to elucidate the significance of the sympathetic fibers by extirpation and stimulation of the superior cervical sympathetic ganglia by Vogt (38), Hinsey and Markee (39), Haterius (40), Friedgood and Pincus (41), Collin and Hennequin (42) and Zeckwer (43) have not been conclusive. Van Dyke (44), in his extensive review of the physiology and pharmacology of the hypophysis, is not impressed with the importance of secretory fibers to the anterior lobe. On the other hand, Roussy and Mosinger (45) stress the part played by the sympathetic system in the neural regulation of the hypophysis.

Innervation of the Hypophysis of Lower Forms

In fish and amphibia there is considerable uncertainty about the nerve supply to the glandular portion. Bockenek (46), Burr (47) and Weinberg (48) found some nerve fibers from the neural lobe penetrating into at least pars intermedia. In amblystoma Herrick (49) observed that some nerve fibers also reached pars distalis, as had been noted by previous authors in urodeles and frogs; but in Necturus (mud-puppy) the tractus hypophyseus ends in the neural lobe where the fibers branch freely with no evidence that any of them enter pars tuberalis, pars intermedia or pars distalis. In well-impregnated Golgi preparations where the neural

lobe is well defined from the rest of the hypophysis, nerve fibers are clearly confined to the former. Since this study on Necturus involved 89 brain series and diligent studies during a period of 25 years, it is improbable that nerve fibers in the glandular lobes would be overlooked, or had not been impregnated in at least some preparations. Herrick found that the tractus hypophyseus of Necturus arises from much of the preoptic nucleus and ventral part of the hypothalamus, and passes into the thin floor and side walls of the infundibulum.

MATERIAL AND METHOD

This report is based primarily on Cajal and Golgi preparations of 47 adult human hypophyses, many of which were cut seriously in various planes. Only one newborn has been studied. The time intervening between death and fixation of the tissue varied from half an hour to several hours. Thirty-two of the hypophyses were prepared by a modification of the pyridine silver method suggested by Dr. Olaf Larsell of the University of Oregon. This brought out the nerve fibers more uniformly than any other technic adequately tried. The Bodian method (21) has been used on only a few specimens. Although some whole hypophyses, both with and without the adjacent cavernous tissue (including internal carotid arteries), were carried through as one block, most of them were cut into 3 or 4 slices to insure more complete impregnation with the reagents. The adjacent tissue was retained in some cases in order to establish the source of the nerves in the capsule of the hypophysis.

The steps in the pyridine silver method which was found to give the best results are listed here because of its usefulness on both normal and pathological specimens where nerve fibers are to be brought out.

Technic. A). Fix small pieces in absolute alcohol containing 6 drops of ammonium hydrate per 100 cc. for 2 to 3 days.

B). Wash in running tap water to get rid of ammonia, 12 hours.

C). 3 per cent nitric acid, 2 hours.

D). Rinse in water a few minutes to an hour depending on the size of the piece.

E). 80 per cent alcohol, several changes, a few hours.

F). 80 per cent alcohol + 4 drops ammonium hydrate to 100 cc. over night.

G). Pure pyridine, 12 hours.

H). Wash out the pyridine with repeated changes of distilled water until no odor of pyridine is detectable.

I). 2 per cent silver nitrate, 24 hours in the dark.

J). ¾ per cent silver nitrate, 24 hours in the dark.

K). 2 per cent silver nitrate, 24 hours in the dark.

L). Rinse hurriedly in distilled water.

M). Reduce in 4 per cent pyrogallic acid, 24 hours.

N). Dehydrate, clear, embed in medium paraffin so sections at least 10 μ thick can be cut.

All illustrations except figure 1 are from 10 μ sections prepared by this technic. All but figures 1 and 2 are from photomicrographs, some of which have been slightly retouched to compensate for the lack of focal depth. Nothing has been added that is not in the area photographed.[1]

[1] The actual specimens were demonstrated at the 54th Annual Session of the American Association of Anatomists, Pittsburgh, Pa., April 14-16, 1938. Anat. Rec. 70:(Suppl. 3)100. 1938. All figures are from adult human hypophyses.

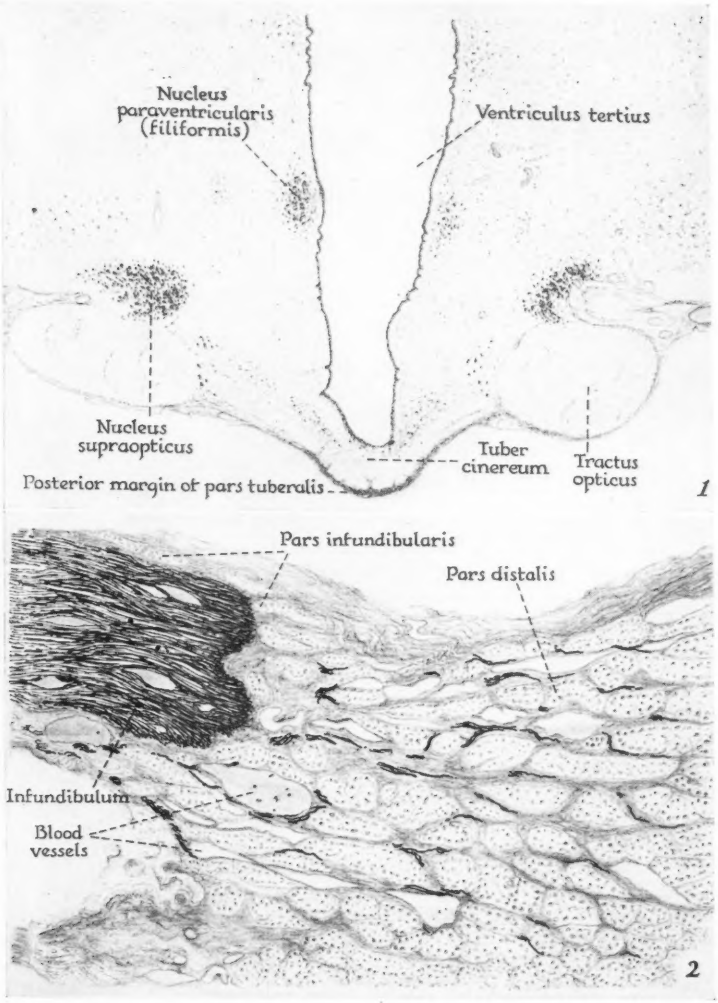
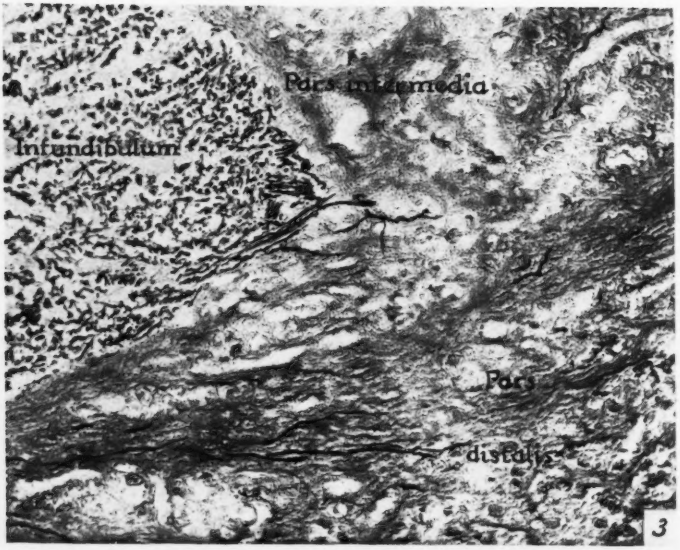


Fig. 1. Camera lucida drawing of a frontal section of the hypothalamic region of an adult human brain just posterior to the infundibulum, showing the location of the supraoptic and paraventricular nuclei. The supraoptic nuclei are the chief source of the nerve fibers that pass into the hypophysis via the infundibulum. 7μ. Cresyl violet.

Fig. 2. Camera lucida drawing of a sagittal section of an adult human hypophysis just lateral to the attachment of the infundibulum, which was slightly bent towards the side of the section so as to be cut obliquely in the upper left corner of the figure. It shows the density of the nerve fibers in the infundibulum and the number of small sympathetic nerves that enter the hypophysis near the stalk in the vascular connective tissue stroma in the upper part of the anterior lobe. 10μ. Pyridine silver.

Golgi and the other silver methods mentioned above have also been applied to the hypophysis of 30 animals (rat, rabbit, guinea pig, cat, dog, monkey). The animal tissue was used as a control on the human material, because animal hypophyses could be fixed while perfectly fresh. This animal tissue appar-



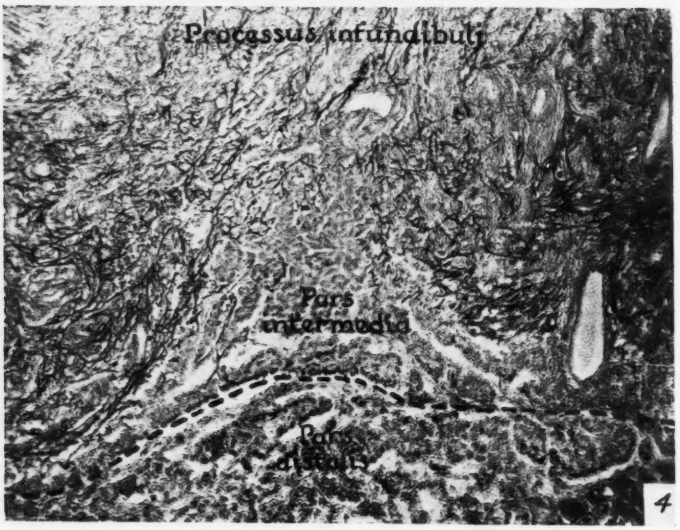


Fig. 3. Horizontal section of lower end of infundibulum and upper surface of anterior lobe (pars distalis) showing nerve fibers from infundibulum passing into pars intermedia. Nerve fibers in the connective tissue passing into anterior lobe are largely, if not entirely, from the sympathetic capsular plexus. 10μ Pyridine silver.

Fig. 4. Mid-sagittal section where pars intermedia is greatly thickened by proliferation of basophilic cells. Abundant nerve fibers are seen in the posterior margin of pars intermedia but none in much of the anterior region. A dense nerve plexus is clearly evident in neural lobe (processus infundibuli). 10μ Pyridine silver.

ently gave no better demonstration of nerve fibers than was obtained from human hypophyses up to 3 and 4 hours postmortem.

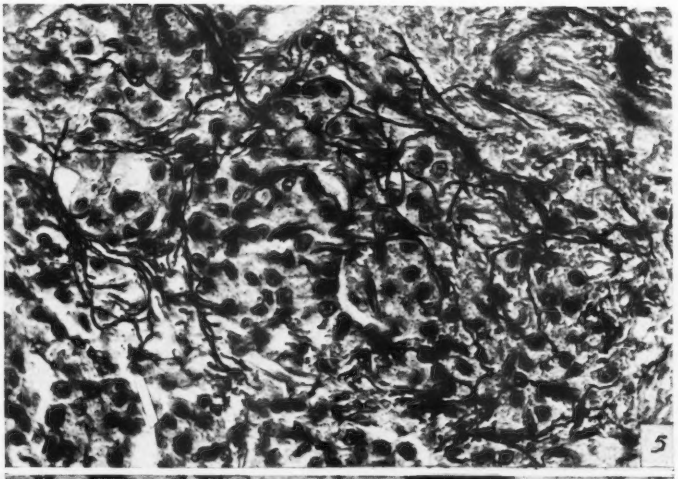
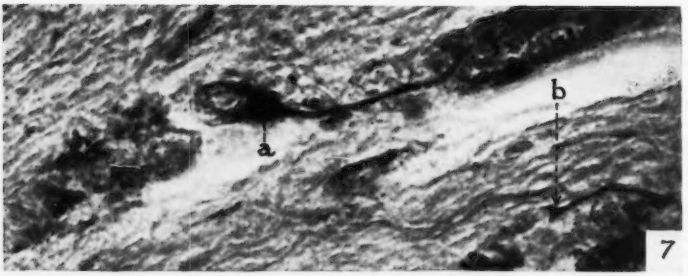


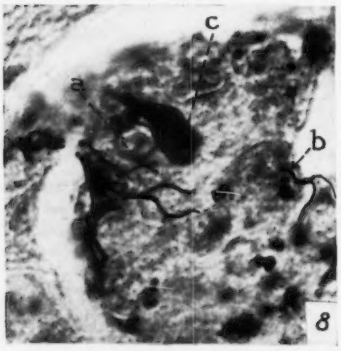
Fig. 5. Higher power view of the nerve plexus in the posterior part of the thickened region of pars intermedia shown in figure 4. Counter stained with hematoxylin to emphasize the cells. 10μ Pyridine silver.

Fig. 6. Sagittal section of typical pars intermedia showing no nerve fibers in the main stratum of epithelial cells but several intertwine in the walls of a vesicle and penetrate slightly into anterior lobe. 10μ Pyridine silver.

RESULTS

The number of fibers in tractus hypothalamicohypophyseus and of cells in nucleus supraopticus. Since some have questioned the magnitude of the hypophyseal tract from the hypothalamus, cross sections of the lower end of 5 well-impregnated stalks were examined under the oil-immersion lens and with a Whipple eye piece micrometer (a glass disc ruled into squares) in the





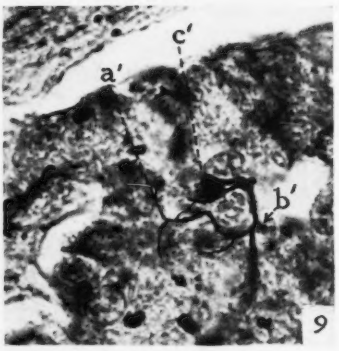


Fig. 7. *a*, nerve terminal in pars intermedia. *b*, 2 fibers cut as they separate to pass around a cell (rest of the course in next sections of series), and hence not a nerve terminal.
Figs. 8, 9. Adjacent sections of apparent nerve terminals in pars intermedia, *a* is continuous with *a'*, and *b* with *b'*; *c* is continuous with *c'* and hence not an enlarged terminal.
Fig. 10. A few nerve fibers between the epithelial cords along the infundibular stem.
Fig. 7-10, 10μ Pyridine silver.

ocular. By means of a mechanical stage the entire area (2 cases) or only those portions where the fibers were cut in cross section (3 specimens) were systematically explored, and the fibers counted. Where only part of the area was counted, the proportion of this area to the entire area was found, and thus the total number of fibers was calculated. The density of fibers in the infundibu-

lum may be appreciated by referring to figure 2 where a longitudinal section of a small portion of the lower end of the stock is shown in the upper left corner. A small portion of a cross section is found in figure 3. Because of clumping of the fibers these illustrations do not give an adequate idea of the small size and great number of fibers involved. For the same reason the estimates given here are more likely to be too low than too high. Slight obliquity is another factor that militates against accuracy. However, the figures are sufficiently accurate to give a general idea of their abundance which ranged from about 40,000 in a newborn to 69,000 in adults. It is interesting to note that in the left supraoptic nucleus of the specimen shown in figure 1 there are nearly 70,000 nerve cells, and in the right about 54,000. The two supraoptic nuclei appear to contain twice as many cells as are necessary to give origin to the fibers that enter processus infundibuli. In the adult these nuclei are about 5 mm. long, and at their greatest diameter contain as many as 200 cells with nucleoli in a single 7 μ section. It would seem, therefore, that the number of nerve cells and fibers connecting the human hypophysis is sufficiently great to merit serious consideration. From a study of similar material from common laboratory animals, including the monkey, the above statement may be said to apply generally.

Distribution of tractus hypothalamicohypophyseus. As the fibers from the supraoptic nuclei converge toward the median eminence of the tuber cinereum, an occasional fiber may stray into the superficial connective tissue and become associated with the epithelial strands, which represent pars tuberalis. Some of these course back into the main bundle; but it cannot be absolutely denied that some may end in this part of the glandular lobe. No very close relationship of nerve fibers with these epithelial cells suggestive of nerve terminals was ever encountered.

The above is essentially true of pars infundibularis, or jacket of epithelial cells that surrounds the infundibular stem. Individual fibers get out into the connective tissue between the epithelial strands as shown in figure 10, which is a longitudinal section of a few of the cell columns well down along the stem. This may be merely an incidental association for some of the fibers can be followed back into the main bundle.

On approaching pars distalis fibers can be followed from the infundibulum into and occasionally through the upper part of pars intermedia as shown in figure 3, which is from a horizontal section and hence transverse to the infundibulum. Most, if not all, of the nerve fibers in the connective tissue of the adjacent pars distalis are not from the infundibulum but from a capsular plexus of sympathetic origin to be described later. The number of infundibular fibers that get into the anterior lobe in this region is very small.

The complexity formed by these thousands of nerve fibers in processus infundibuli is difficult to describe. Figure 4 gives only a very imperfect idea of the tangled mass of fibers in a 10 μ section. The number of nerve fibers would seem to be out of all proportion to the number of cells present, were the neuroglia-like cells (pituicytes) proven to be secretory. Neither would such a wealth of fibers be necessary for innervating pars intermedia. In some human hypophyses, pars intermedia represents less than half a milligram of tissue

(50), consisting largely of a single layer of cells and even being completely lacking over considerable areas of the adult organ. One is almost forced to assume that the hormones produced by the neural lobe are liberated without the intervention of secretory cells in the usual sense, as in the humoral mechanism associated with the sympathetic nervous system. The density of the nervous network appears to be such that it would be virtually impossible to disprove on morphological grounds functional relations between the nerve fibers and the cells found within their meshes. Brooks and Gersh (34) have just reported that there are pericellular nerve terminals in the neural lobe of the rat. We have been unable to confirm this.

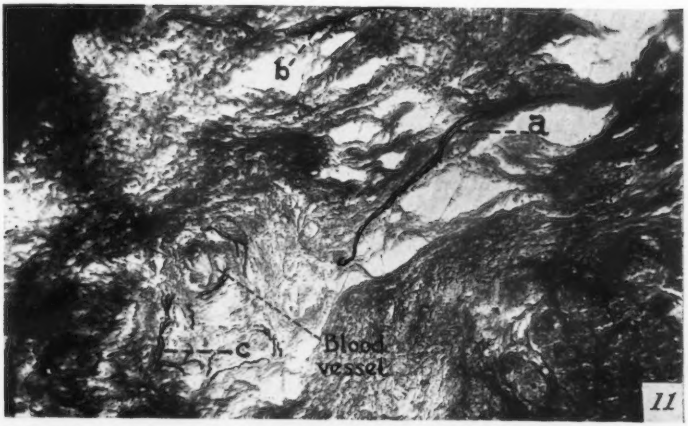
Here and there some of these nerve fibers penetrate into pars intermedia and intertwine among the epithelial cells as in figure 6. Many of these fibers appear to be merely traversing this epithelial stratum rather than terminating there. Most of those shown in the walls of the vesicle in figure 6, when followed in serial sections are passing around the vesicle and going beyond it into the adjacent anterior lobe and more remote portions of pars intermedia. As is shown well in figure 6, the main lamina of cells in pars intermedia may be quite free from demonstrable nerve fibers for large stretches. Where pars intermedia is represented by masses of basophilic cells which invade the neural lobe, as in figure 4, these basophilic cells may be enmeshed in a rich nerve plexus as shown in figure 5, which is a higher power view of the posterior border of the thickened portion of pars intermedia of figure 4. The anterior portion of such a region of pars intermedia may be free from nerve fibers. In short, the cells of the intermediate lobe appear to have invaded the nerve plexus of the neural lobe so that the intermingling of the two structures may be only secondary. The whole range from no nerve fibers in vast areas of pars intermedia to an extremely rich plexus of nerve fibers may be observed in the same gland. There seems to be a great deal of haphazardness in the arrangement of the nerve fibers encountered in pars intermedia.

In the intermediate region of the human hypophysis many appearances simulating nerve endings on and within the epithelial cells are encountered as *b* of figure 7, *c* of figure 8 and *c'* of figure 9. In serial sections these can usually be shown to be points where two or more nerve fibers are cut by the microtome as they pass obliquely to a higher or lower level as they pass around a cell. Thus *b* of figure 7 is merely a point where two fibers separate as they approach a cell. The rest of the course of the fibers is in the adjacent sections. This might not have been suspected from the single section. In figure 8, *c* is a bundle of fibers coming obliquely over a cell and is cut off just below the upper surface of the cell. The next section is shown in figure 9, *c'* being

Fig. 11. Tangential section through capsule on upper surface of anterior lobe showing a typical nerve plexus, which is derived from the cavernous plexus. The nerves near *c* descend into the anterior lobe just lateral to the attachment of the infundibulum, and are usually associated with blood vessels. *a*, *b*, small nerves that descend in the capsule and enter the gland at various levels. 10μ Pyridine silver.

Fig. 12. Oblique section through a robust connective tissue trabecula near the upper surface of the anterior lobe containing an unusually large bundle of nerve fibers which have come from the capsular plexus shown in figure 11. One of the several accompanying veins is shown in the lower right corner. 10μ Pyridine silver.

Fig. 13. Oblique section of an artery in a connective tissue trabecula deeper in the anterior lobe showing associated nerve fibers, which largely follow the branches of the artery. 10μ Pyridine silver.



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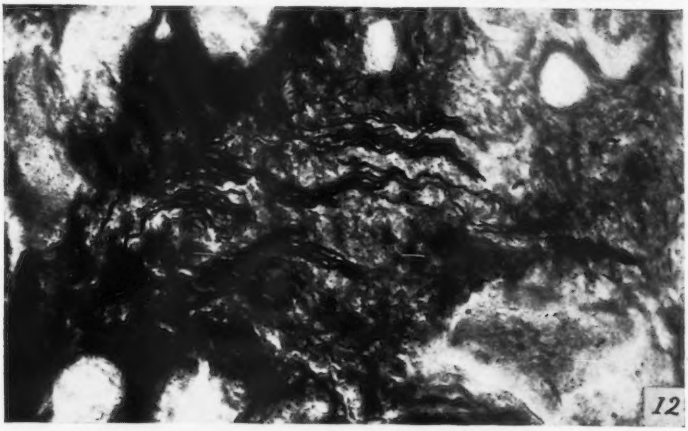

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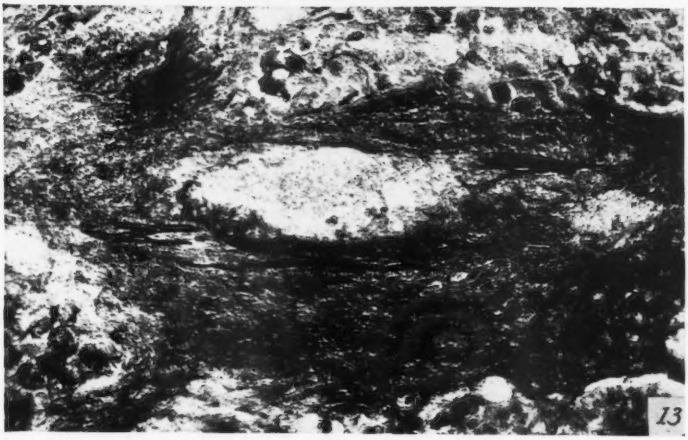

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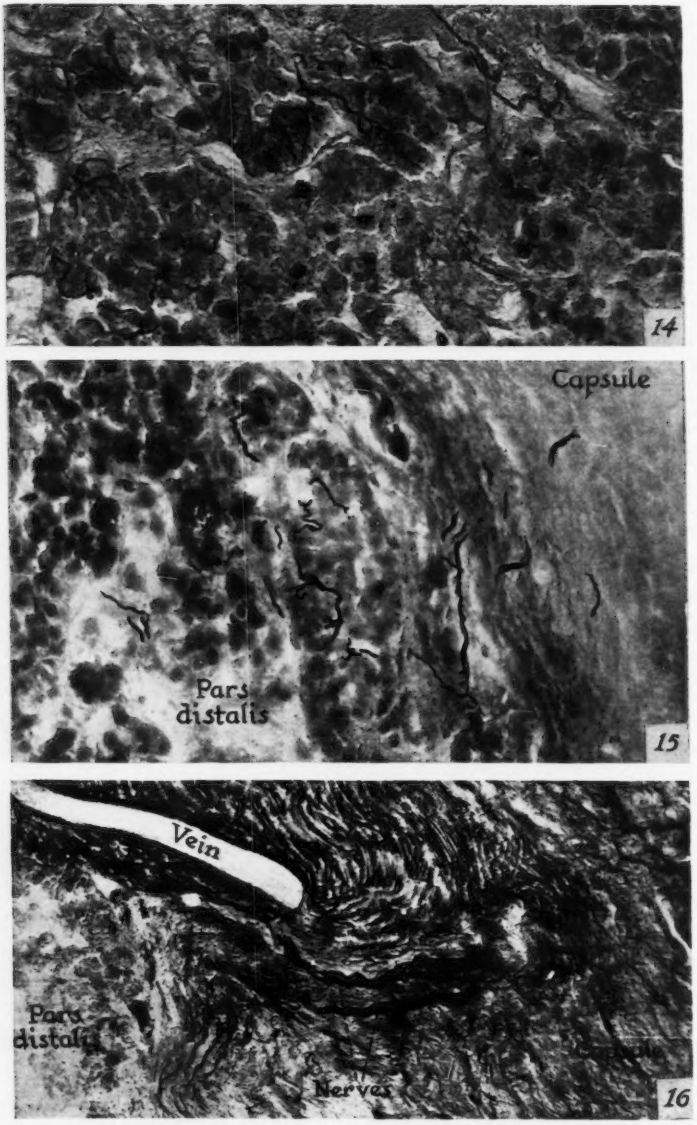

Fig. 14. Nerve fibers deep in the anterior lobe. 10μ Pyridine silver.
Fig. 15. Nerve fibers from the capsular plexus entering the anterior margin of anterior lobe.
Fig. 16. Nerves in the capsule following a vein into the region between anterior and posterior lobes. 10μ Pyridine silver.

a continuation of *c* (fig. 8). Here the individual fibers can be seen as they leave the cell to converge again to a more compact bundle. *b* of figure 8 is connected with *b'* of figure 9, and *a* of figure 8 is continued as *a'* of figure 9. Too often individual sections are interpreted quite independently of adjacent

sections in which the remainder of a structure may be found, the third dimension being largely ignored.

A few endings in pars intermedia cannot be thus discounted. In figure 7, *a* is evidently the same as the terminals described by Tello (22). The nerve fiber cannot be followed beyond the cell in this or adjacent sections; but it is not clear that the dark expansion against the nucleus of the cell is a part of the nerve fiber, as assumed by Tello. One end of many cells in over-impregnated regions of Cajal preparations may be very dark brown or nearly black. Were a nerve fiber to come close to such a cell, it might appear to enter the darkened part of the cell. In the one shown in figure 7, and in a few others, the nerve fiber appears to be continuous with the dark expansion; but it still remains to be clearly demonstrated that the nerve fibers actually enter the cells as maintained by Tello. Granting that there are a few actual nerve terminals of this sort, their scarcity militates against their being secretory since, if secretory, one would expect a relatively large proportion of the cells to be thus supplied, although the degree of intimacy between gland cells in general and nerve fibers is far from settled. Many mistakes have arisen from a failure to realize the limitation of the technic used. The tendency for reticular connective tissue to stain with certain silver methods, and especially the much-used Bielschowsky technic (51, 52) has been particularly disturbing, and has overemphasized the abundance of nerve tissue in the proximity of the secreting cells of various glands.

The origin and course of the capsular nerves. A number of small nerves composed of unmyelinated fibers are regularly present in the connective tissue capsule of the hypophysis. They come from the cavernous plexus, mostly following along the hypophyseal vessels. They are most abundant on the upper surface of the anterior lobe, from which figure 11 was taken. A considerable number of these fibers (the ones marked *c* in fig. 11) pass into the anterior lobe and spread out anteriorly and laterally along the connective tissue trabeculae and accompanying veins of the upper superficial region as shown in figure 2, which is a sagittal section. The nerve fibers become more and more scattered until individual fibers may be seen in the vascular stroma between cell cords. Here and there a fiber may be woven in and out among the epithelial cells.

A prominent bundle of as many as 50 fibers, as shown in figure 12, frequently descends for a considerable distance into the interior of the anterior lobe commencing just lateral to the infundibulum. It usually becomes associated with an artery (fig. 13) and several prominent veins. Some of these fibers finally become disseminated among the cell masses in a limited region deep in each half of the lobe, as shown in figure 14. While nerve fibers are regularly demonstrable in the interior of the anterior lobe, they are confined to very limited regions.

Small nerves, such as *a* and *b* of figure 11, pass forward and downward in the capsule, frequently near the larger blood vessels. As many as seven such bundles of nerve fibers may be encountered in a single horizontal section through the middle of the gland. At intervals a few fibers enter the parenchyma as shown in figure 15. Occasionally a nerve as large as shown in figure 16 follows a blood vessel into the gland. The one illustrated in figure 16 is located

in the groove between the two main lobes and follows a large vein, sending branches into both the anterior and posterior lobes. The fibers entering the posterior lobe become lost in the dense neural plexus already there, while those that proceed into the anterior lobe are rapidly dispersed in the lateral margin of the parenchyma. Thus at numerous points the superficial part of pars distalis is permeated by a few nerve fibers that in general can be followed only a short distance into the glandular substance. The more prominent bundles of fibers are closely associated with the blood vessels, but individual fibers stray off into adjacent cell cords. However, nothing that could be definitely regarded as nerve endings on the gland cells of this lobe has ever been seen in this material. The varicosities mentioned by others are so common on unmyelinated fibers that they may be disregarded. They occur along the nerve fibers as they course along in connective tissue, with which they have no functional connection. Some knob-like endings are nothing but points where a fiber makes a sudden bend and is cut off as it proceeds more or less vertically to the plane of section. The end view of the cut fiber plus the kink at the bend may appear to be a terminal knob.

Small bundles of nerve fibers from the capsular plexus have been followed up along the blood vessels of the stem. We have no good silver material with a sufficient amount of the tuber cinereum attached to follow these thoroughly. They are most likely, to a large extent at least, vasomotor.

SUMMARY

The literature on the nerve supply to the hypophysis shows that considerable disagreement still exists on many points. Data on the nerves of the human gland are very meager and yet of the utmost importance.

The original work is based on the study of 48 human hypophyses, prepared by selective silver methods, applied to the whole organ as well as to blocks of various thicknesses and cut in various planes. As a check on what was lost by postmortem changes in the human material many fresh hypophyses from animals (rat, guinea pig, rabbit, cat, dog and monkey) were similarly treated. Nerve fibers are as conspicuous in the hypophysis of man as in any of these animals. Further generalizations made here refer specifically to the human material, although they apply in a general way also to the animals.

There are at least 50,000 fine unmyelinated nerve fibers descending from the hypothalamus into the infundibulum, and there are that many cells in one supraoptic nucleus. This relatively enormous nerve supply appears to be all out of proportion to the cellular content of the neural lobe, and to the amount of epithelium in pars tuberalis, pars infundibularis and pars intermedia.

The density of nerve fibers in processus infundibuli (posterior or neural lobe in a narrow sense) renders it virtually impossible to rule out by morphological means the possibility of nerve contacts with the cells in its meshes. However, these cells have never been proven to be secretory, and the nerves may function through a humoral mechanism rather than by direct activation of secretory cells.

Only an occasional nerve fiber is seen among the epithelial cells of pars

tuberalis and pars infundibularis (the jacket of cells along the infundibular stem.

Large areas of pars intermedia apparently may be devoid of nerve fibers, whereas in other places, especially where it is represented by basophilic cells that invaded the neural lobe, there may be a rich plexus of nerve fibers between many of the epithelial cells, but this evidently is a case of epithelial cells having grown into a nerve plexus and hence of no primary importance. Many of the nerve fibers encountered in pars intermedia appear to be there only incidentally. There are a number of structures that suggest nerve terminals on the cells of pars intermedia. Many of these are apparent rather than real. A few cannot be thus disposed of.

A few nerve fibers pass from the neural lobe through the intermediate region and get slightly into pars distalis (anterior lobe), but the number is negligible.

There are numerous small unmyelinated nerves in the connective tissue capsule of the hypophysis. They are most conspicuous on the upper surface of the anterior lobe. They are derived from the cavenous sympathetic plexus and probably represent about 200 fibers. A few of these ascend along the blood vessels of the stalk. A conspicuous number pass into and along the vascular stroma anteriorly and laterally in the upper superficial part of the anterior lobe. A few individual fibers ultimately ramify among the epithelial cells.

A large right and a left group of fibers, commencing just lateral to the stalk, descend deeply into the corresponding half of the anterior lobe, where they usually become associated with prominent blood vessels. Some of these fibers disseminate themselves among the gland cells of a limited region deep in the anterior lobe.

Several small capsular nerves pass downward and forward, sending a few fibers into the substance of the anterior lobe at numerous points. The larger strands are usually near arteries or veins. Individual fibers from this source intertwine among the gland cells, but rarely penetrate very far into the lobe. No definite nerve terminals were seen strictly in the anterior lobe. Some of the capsular fibers have been followed along blood vessels into the neural lobe they become lost in the dense nervous plexus.

Such a large proportion of the anterior lobe appears to be so devoid of nerve fibers that secretory nerves to this part of the hypophysis remain highly questionable. The nerve fibers in this lobe are most likely connected with the vascular system.

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CHANGES IN THE HYPOPHYSIS AND THE OVARIES OF RATS CHRONICALLY TREATED WITH AN ANTERIOR PITUITARY EXTRACT

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That chronic treatment with hypophyseal extracts may cause morphological changes in the anterior lobe of the pituitary was first observed by Bell (1) in the guinea pig, in which the chromophil granules disappear under the influence of such treatment. But even before that Berblinger (2) found an increase in the weight of the hypophysis of rabbits treated with gonadotropic extracts of placental origin. He observed that such treatment causes the development of 'pregnancy cells,' a finding which has subsequently been confirmed in various species by numerous investigators (3 to 9).

Collip, et al (10) and Thomson, et al (11) noted in the course of their experiments with thyrotropic anterior lobe extracts that, under the influence of chronic treatment with such preparations, the ovaries of rats involute, corpus-luteum formation ceases, and at the same time castration cells and cysts form in the anterior lobe of the hypophysis. Fluhmann (12) confirmed these findings, using a sheep anterior lobe extract, which led to considerable atrophy of the ovaries accompanied by the formation of numerous castration cells in the hypophysis. It is important, however, to distinguish between two distinctly different stages in the effect of both the gonadotropic preparations extracted from pregnancy urine or placenta and those prepared from the hypophysis. In both cases there is a first stage during which the ovaries are enlarged and contain many corpora lutea, whereas the hypophysis resembles the condition of the pregnancy pituitary in its histological appearance. Under the influence of prolonged treatment, the ovaries atrophy (13, 14) and, at least in the case of treatment with hypophyseal extracts, castration cells appear in the hypophysis.

It is known that the transformation of theca cells into so-called 'wheel cells' (15, 16) is a characteristic sign of hypophyseal deficiency which disappears under the influence of treatment with relatively small doses of the gonadotropic hormones. That the formation of wheel cells is really an extremely sensitive indicator of the sex hormone production is well shown by the fact that in the immature rat, in which the hypophysis does not produce enough gonadotropic hormone to allow the formation of mature follicles and corpora lutea, there still is enough gonadotropic hormone in the circulation to prevent the formation of wheel cells. Yet if such immature rats are deprived of their hypophysis, wheel cells form within a short time, thus proving that the immature hypophysis, although unable to cause ovulation, does exert a constant trophic effect on the ovary (17).

It seemed of interest, therefore, to establish whether by continuous treatment with a pituitary preparation one could cause such a marked atrophy of the gonads that wheel cell formation would result. For this purpose we performed a series of experiments in which rats were injected twice daily subcutaneously with 0.04 cc. of a pituitary extract prepared from the dissected acetone-defatted anterior lobes of pig glands. After treatment for 4 hours, at room temperature with dilute alkali pH 10, the mixture was neutralized to pH 5 and filtered. The filtrate was concentrated at low temperature and pressure

TABLE 1. RATS 21 TO 30 DAYS OLD

Length of Treatment weeks	No. of Animals	Wt. of Ovaries mg.
1	17	97
2	18	113
3	24	104
4	18	222
5	18	197
6	18	152
7	18	142
8	17	138
10	12	151
11	18	118
13	12	61
15	18	89
24	6	77

TABLE 2. RATS 2½ MONTHS OLD

Length of Treatment weeks	No. of Animals	Wt. of Ovaries mg.
1	6	97
2	6	136
3	8	112
5	8	91
7	6	47
9	6	77
10	6	58
13	6	89[1]
16	3	24

[1] The average weight of the ovaries of 5 animals in this group was 30 mg. The ovaries of the 6th animal weighed 383 mg.

to a total solid content of 2.8 per cent; 10 per cent alcohol was added as a preservative. It was rich in both gonadotropic and thyrotropic principles: 0.01 cc. administered thrice daily to 21-day-old female rats gave ovaries of 44 mg. in 72 hours; 0.02 cc. administered twice daily to 200-gm. guinea pigs gave thyroids of 37 mg. which, on histological examination, were definitely hyperplastic.

At varying intervals some of the animals were killed, and their ovaries weighed. At the beginning of our experiment the rats in the 1st series were 21 to 30 days old (average 26) and those in the 2nd series 2½ months of age. The results are summarized in tables 1 and 2. From these data it is evident that although the treatment first increased the size of the ovaries, later a decrease of ovarian size ensued, the peak of the curve being reached some-

what sooner in the adult than in immature rats. In the immature animals, however, the absolute weight of the ovaries rose to a higher level than in the adults. From this it would appear that resistance to this treatment develops more readily in the adult than in the immature female. In a 3rd series, female rats aged 8 to 11 weeks were hypophysectomized and 5 days later injected with the same extract and in the same manner as the animals of the above

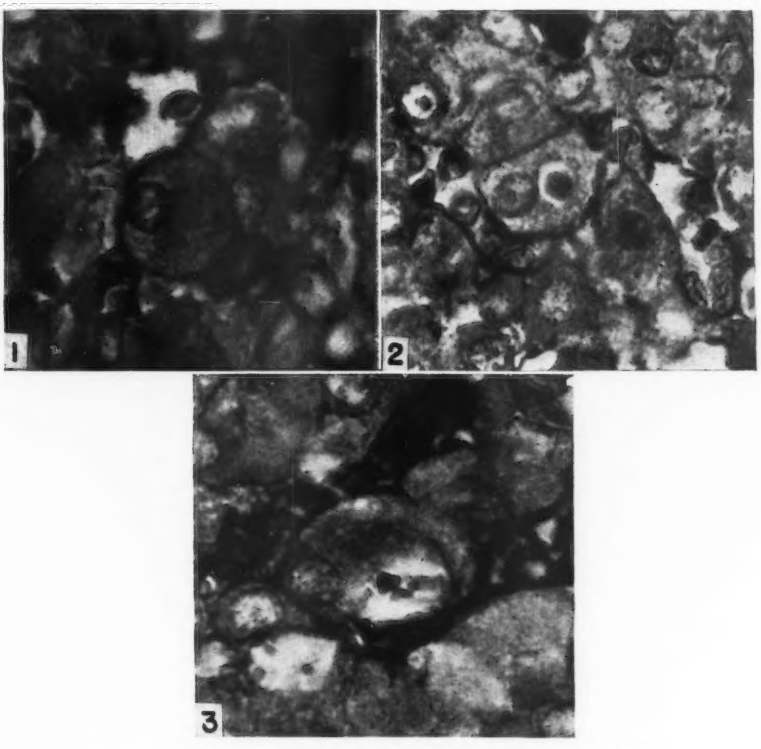

Fig. 1, 2, 3. Three successive stages in the formation of a 'signet ring' cell in the hypophysis of a rat treated for 8 weeks with a pig pituitary extract.

series. Resistance developed, although less rapidly than in the normal rats (table 3).

Histological examination confirmed our previous findings concerning the formation of castration cells and cysts in the hypophysis, and showed that such changes may occur at any time after the 4th week of treatment. Three successive stages in the formation of the characteristic 'signet ring' castration cells are shown in figures 1, 2 and 3. In many of these pituitaries the vacuolated signet ring cells degenerated, and we saw numerous transitional stages between areas in which 2 or 3 of these vacuolated cells melted together after liquefaction, and true cysts resulting from the confluence of a large number of these liquefied degenerated elements. The cells of the surrounding parenchyma

usually arrange themselves so as to form a smooth lining around these cysts.

There is a fairly close parallelism between the appearance of the hypophysis and that of the ovary, in so far as castration changes were only seen in animals whose ovaries had begun to show signs of involution and were free of recent corpora lutea. This does not mean, however, that the ovaries had to be subnormal in size before castration cells developed in the pituitary. In many of our cases the ovaries were still considerably above normal in their gross weight at this time, but histologically all the corpora lutea proved to be involuting and there were no mature follicles or corpora lutea. At a somewhat later date after the 8th week of treatment, typical wheel cells appeared

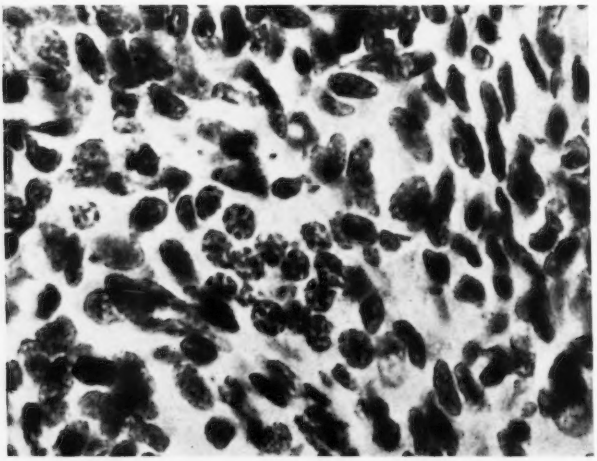

Fig. 4. Wheel cells in the ovarian stroma of a rat treated for 13 weeks with a pig pituitary extract.

in the ovaries of many animals (fig. 4) and these were most pronounced in those cases in which the castration changes in the hypophysis were particularly severe. It became obvious that the degree of ovarian atrophy is directly proportionate to the degree of castration cell formation.

These experiments seem also to be of significance for the theory of antihormone formation as outlined by Collip (18). It would be easy to understand that after a certain time, the ovaries become refractory to the injected hormone but, unless this refractoriness also inactivates the gonadotropic effect of the animal's own hypophyseal secretion, it is difficult to understand why their ovaries should actually be atrophic, that is to say, less developed than those of untreated animals—in fact, even less developed than the ovaries of immature rats.

An experiment in which, after 16 weeks of treatment with the pig pituitary extract, resistant rats of this series were implanted daily with 2 pituitaries of normal rats showed that these animals were not only resistant to the pig pituitary extract with which they were pretreated but also, at least to some degree, to the gonadotropic principle of rat hypophysis. One of these animals

received pituitary implants during 8 days after an exploratory laparotomy had established that the ovaries became atrophic and resistant to the action of the pig pituitary extract. On the 9th day, the animal was killed. Its ovaries were approximately the same size as at the time of inspection before beginning implant treatment. Histologically, it proved to be free of wheel cells but contained no mature follicles or corpora lutea. A 2nd animal received 12 days' treatment with rat pituitary implants in a similar manner. Here again, there

TABLE 3. HYPOPHYSECTOMIZED RATS

Length of Treatment weeks	No. of Animals	Wt. of Ovaries mg.
2	7	98
3	7	112
4	5	122
5	6	108
6	5	147
7	4	94
9	4	61
12	4	88
13	5	59

was no visible enlargement of the gonad as compared with its appearance at the exploratory laparotomy before implantation. Histologically, the structure of the ovary was identical with that of the animal treated for 8 days. The pituitary of both these animals showed castration cells. Although the absence of wheel cells and a very slight degree of theca luteinization seemed to indicate that the ovary was not entirely resistant to the implants, the absence of follicle maturation and corpus-luteum formation indicates that pretreatment with the pig pituitary extract undoubtedly decreased its sensitivity to the gonadotropic principle of rat pituitary. In a 3rd animal, in which the ovary was likewise examined by laparotomy at the end of the 16th week of treatment with the pig pituitary extract, and found to be greatly reduced in size, 15 daily implants with 2 rat pituitaries resulted in no visible change in the size of the gland as far as one could tell at the occasion of a second laparotomy performed at the end of this treatment. In this case, however, we did not kill the animal but continued treatment during a period of 7 days with 0.08 cc. of the above-mentioned pig pituitary extract in combination with 100 R.U. of a gonadotropic extract prepared from pregnancy urine. At the end of this time, the animal was killed. Its ovaries were greatly enlarged but contained no visible corpora lutea. Histological examination showed no trace of follicle maturation or corpus luteum formation but the theca around the unaltered immature granulosa of the follicles showed the typical picture of theca luteinization. This response is the characteristic reaction of hypophysectomized or extremely immature animals treated with gonadotropic extracts of pregnancy urine (19, 20, 21).

SUMMARY

The experiments reported show that continuous treatment with a pig pituitary extract results in a transient increase in ovarian weight due to the

formation of many corpora lutea. This increase reaches its peak more rapidly in the adult rat, but in the immature animal the increase is more pronounced and reaches a much higher level. From this it appears that resistance to such treatment develops more rapidly in the adult than in the immature female. In hypophysectomized rats, the peak of the curve is somewhat delayed in comparison with the normal, mature or immature animal, but finally resistance develops even in the absence of the hypophysis, and the ovarian size retrogresses. Parallel with the development of ovarian atrophy, 'signet ring' cells appear in the hypophysis, and cysts form in some cases as a result of liquefaction and confluence of these vacuolated cells.

At the height of this acquired resistance, the ovary shows such severe atrophy as is only observed in hypophysectomized animals, and the characteristic 'wheel cells' appear. These ovaries are not only resistant to the gonadotropic action of the extract used but also, at least to some degree, to implants of rat hypophysis. Both these facts indicate that our treatment led to a condition of at least relative immunity to the animal's own hypophyseal secretion. This interpretation received further support by an experiment in which massive doses of a gonadotropic pregnancy urine preparation failed to cause follicle maturation or corpus-luteum formation, but led exclusively to pronounced theca luteinization. This is otherwise never observed in adult rats treated with such preparations, except after the complete surgical removal of the hypophysis.

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HORMONE STUDIES WITH THE ULTRA-CENTRIFUGE

II. THE CONCENTRATION OF ANTERIOR LOBE AND PITUITARY-LIKE HORMONES WITH THE ULTRA-CENTRIFUGE[1]

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The purpose of this paper is to describe a first group of experiments designed to test the possibility of concentrating and separating the anterior lobe and pituitary-like hormones by means of the ultra-centrifuge. Preparations of castrate (menopause) urine, pregnant mare serum and flavianate extract of beef hypophysis were used. In a preliminary run with a solution of thyroglobulin, it was found that after 2 hr. at 600 r.p.s. practically all of the thyroglobulin had been centrifuged into the bottom cubic centimeter of each tube's contents. It was therefore arbitrarily decided to use speeds of 600-700 r.p.s. for 4 to 6 hr. in the attempt to concentrate the gonadotropic hormones. Table 1 shows the centrifugal fields at the top and bottom of the centrifuge tube at various speeds.

METHODS

Experiment 1. Castrate urine preparation. L456cx. 1 cc.=⌔=0.444 mg. =⌔=3.33 M.U. Fifty cc. of this preparation were centrifuged at 615 r.p.s. for 4 hr., at the end of which run the centrifuge was allowed to coast to a stop in 3½ hr. By means of a 5 cc. Luer syringe, the contents of the 10 tubes were separated into 5 fractions. The upper ⅓ of each tube was pooled to form fraction 1. The middle ⅓ of each tube constituted fraction 2. The bottom ⅓ of 5 tubes composed fraction 3. The bottom fraction in the remaining 5 tubes was further subdivided, the upper ⅔ of each being pooled to make fraction 4, and the bottom ⅓ constituted fraction 5.

These 5 fractions were tested for gonadotropic potency by injecting them into 20 to 22-day-old female mice. Three consecutive daily injections of 0.3 cc. were made and the mice autopsied on the following day. Each mouse received 0.90 cc. of injected substance which before centrifuging was equivalent to 3 uterine M.U. From an examination of figure 1 it will be seen that the top fraction 1 had a potency of approximately 1 uterine M.U., a loss of ⅔ of its potency. The bottom fraction 5, on the other hand, had more than doubled in potency. A definite concentration of the hormone was obtained in the lower fractions.

[1] Aided by a grant from the Committee on Research in Problems of Sex, The National Research Council, administered by Philip E. Smith.
[2] E. R. Squibb and Sons Fellow.

A brown gelatinous pellet formed in the bottom of each tube. These pellets were dissolved and injected alone and with specific doses of the upper fraction to test for gonadotropic potency or augmentation of potency. The pellets were found to be without demonstrable potency, and are therefore assumed to be inert material.

Experiment 2. Castrate urine preparation. L456cyl. 1 cc.=⌬=0.444 mg. =⌬=3.333 M.U. A second run was made with another portion of this same castrate urine preparation, but the speed of 650 r.p.s. was maintained for 6½ hr. before the centrifuge was allowed to coast to a stop. The total elapsed time of run and coast was more than 10 hr. The material in each tube was divided into 5 fractions of 1 cc. each, and similar fractions were pooled. In the upper 4 cc. of each tube the potency had fallen uniformly to about 1½ M.U. per cc. The potency of the bottom cc., however, had increased from 3.3 M.U. to approximately 10 uterine M.U. The results are shown on the graph in section 1, II of figure 1. The ovarian and uterine weights are strikingly uniform.

Experiment 3. Castrate urine preparation L456cy. 1 cc.=⌬=20.0 mg. =⌬=150 M.U. In this experiment a more concentrated solution of the hormone used in the previous run was subjected to 100,000 g.[3] for 6½ hr. The contents of each 5 cc. tube were divided into 3 fractions, an upper of 1¼ cc., a middle of 2¾ cc. and a lower of 1 cc. The upper fraction was clear, the middle fraction a light amber which progressively deepened below into the dark brown of the bottom fraction. A large brown gelatinous-like pellet formed in the bottom of the tube. It was probable that, if centrifuging had been continued, all of the colored substance would have been concentrated into pellet form. This pellet was tested for gonadotropic activity and found to be negative, confirming previous assays.

The 3 fractions were injected into 22-day-old mice in varying dilutions to assay their potencies. It was found that the top fraction retained less than 25 uterine M.U. per cc. The middle fraction assayed about 80 M.U. per cc. and the bottom fraction about 400 M.U. per cc. This hormone concentration is similar to that obtained in the previous runs with more dilute preparations.

Experiment 4. Antex (pregnant mare's serum). 491. 1 cc.=⌬=1 mg.=⌬= 5 M.U. (label assay). In this experiment a preparation of Antex which, according to the manufacturer's statement, assayed 5 M.U. (ovarian) per cc. was centrifuged at 650 r.p.s. for 6 hr. The solutions remained clear throughout. A slight jelly-like pellet formed in the bottom of the tubes. The contents of each tube were divided into 5 fractions of 1 cc. each and similar fractions pooled. The potency of each fraction was assayed on 3 groups of mice, which received total doses of 0.3 cc., 0.6 cc., and 0.9 cc. per mouse, respectively. An examination of the data, graphically presented in figure 1, III, shows that a marked concentration of the gonadotropic principle has been obtained in the bottom fraction. Ovarian weights averaged above 18 mg. for the bottom fraction as compared with 5 mg. for the top fraction.

The top and bottom fractions were subsequently further diluted for

[3] g. = gravity.

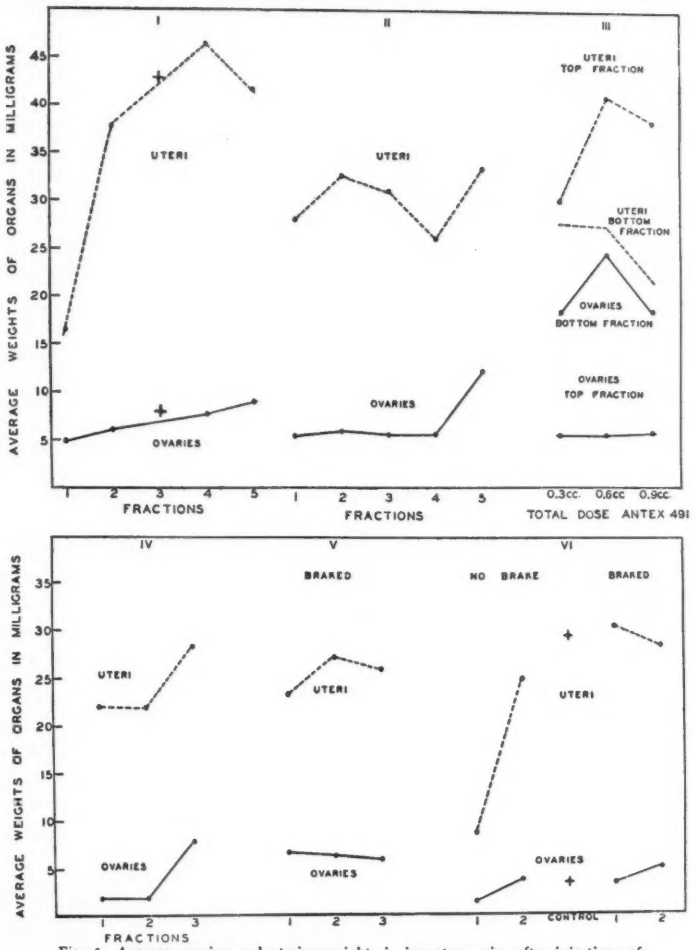

Fig. 1. Average ovarian and uterine weights in immature mice after injections of various centrifuged gonadotropic hormones.

more accurate determination of the minimal stimulating dose. With the bottom fraction, doses calculated on the basis of original content to be 0.45 M.U. actually produced ovaries averaging 6.2 mg. and uterine weights of 23.1 mg. This response would be expected with the injection of from 2 to 3 M.U., suggesting a hormone concentration of 4 to 6 times that of the original preparation.

The pellets from all 10 tubes were collected and dissolved in 10 cc. of water and injected in varying doses. Even in massive doses (1.8 cc.) no indication of ovarian or uterine stimulation was evident.

Experiment 5. Antex. 505. 1 cc.=10 M.U. This preparation was centrifuged at 700 r.p.s. for 6 hours to test the effect of a greater g. (125,000) on the concentration of the gonadotropic hormone. Three equal fractions were

separated and each injected in total doses of 0.3 cc.=3.3 uterine M.U. Ovarian and uterine weights are plotted in figure 1, IV. The upper and middle fractions show similar low potencies of about 1 M.U. per cc., a loss of more than 2 M.U. per cc. The bottom fraction shows a marked rise in gonadotropic potency. It seems not unreasonable to assume that, with longer centrifugation at higher speeds, all the potency could be segregated into the lower fraction.

The effect of braking on concentration of substances of low molecular weight. In all the previous experiments the centrifuge was allowed to coast to a stop at the completion of a run. This took from 3½ to 4 hr., and was often a considerable inconvenience. An air brake, designed by one of us (Chiles), and described in a previous paper,[4] permitted deceleration of the centrifuge from a speed of 700 r.p.s. to a full stop in 20 min., approximately the same time as is required to bring the centrifuge from rest to that speed

TABLE 1

Speed r.p.s.	Centrifugal field at top of fluid (g.)	Centrifugal field at bottom of fluid (g.)
600	52,000	101,000
650	61,000	119,000
700	71,000	138,000
750	82,000	159,000
800	93,000	180,000
850	105,000	204,000

with 30 lb./sq. in. driving pressure. A preparation of Antex was centrifuged and the brake used. Subsequent assays showed that no concentration of the hormone had been obtained. The failure to repeat previous successful concentrations was attributed to the use of the brake, since all other experimental factors had been kept constant. Hence, a series of 5 test runs were made on an aqueous solution of colloidal iron in order to study the stirring effects of braking on the contents of the rotor.

Experiment 6. Colloidal iron. In the 1st run the rotor was operated at a speed of 650 r.p.s. for 30 min., after which the power was shut off and the machine allowed to coast to a stop without using the brake. About 4 hours were required for the rotor to come to rest.

On examining the contents of the tubes, it was found that from the meniscus to about ⅝ inches below it the fluid column was completely clear, and apparently contained no colloidal iron at all. At the bottom of this clear portion there appeared a definite though diffuse boundary, below which the color of the liquid was a light yellow that became darker toward the bottom of the tubes. At about ¼ inches from the bottom of the tubes was a very sharp boundary, below which the color was a dense dark red. Most of the colloidal iron had been thrown down into this region.

In the 2nd run, the rotor was operated for 1 hr. 40 min. at 650 r.p.s., after which an air pressure of 20 lb./sq. in. was applied to the braking turbine. This brought the rotor to a stop in about 20 min.

Examination of the tube contents showed no clear portion below the

[4] Chiles, J. A., Jr., and A. E. Sevringhaus: J. Exper. Med. 68:1. 1938.

meniscus, a light yellowish color beginning at the top of the liquid column and becoming darker toward the bottom. In general, however, the density of this yellowish portion seemed to be no greater than the density of the yellowish portion between the upper and lower boundaries in the preceding run. At about ¼ inch from the bottom of the tubes there appeared a boundary as sharp as in the preceding run, below which the color was a dense dark red. There was also found at the bottom of the tubes a pellet of colloidal iron thrown out of suspension.

In the 3rd run the rotor was operated at 650 r.p.s. for 40 min., after which a pressure of 20 lb./sq. in. was applied to the brake until the speed

TABLE 2

	Volume	Total solids	Solids per cc.	Activity mg./M.U.	Total M.U.	M.U. per cc.
Uncentrifuged original solution L521	25 cc.	500 mg.	20 mg.	0.4	1250	50
Centrifugate						
Top fraction L521B	19 cc.	191 mg.[1]	10 mg.	0.4	480[1]	25
Bottom fraction L521C	3.5 cc.	132 mg.[1]	38 mg.	0.4	330[1]	86
Jelly-like pellet L521A					<100	

[1] A portion lost during manipulations.

had fallen to 300 r.p.s. The brake was then turned off and the rotor was allowed to coast freely to a stop. The time during which the brake was applied was about 10 min., and the time required for the rotor to coast from 300 r.p.s. to a stop was about 3 hr.

The results of this run were practically identical with those of the first run, the completely clear portion again appearing at the top of the liquid column.

In the 4th run the rotor was operated at 650 r.p.s. for 40 min., after which there was applied to the brake a continuous pressure of 1 or 2 lb./sq. in. that brought the rotor to a stop in about 1 hr., 30 min. The results of this run were about the same as those obtained in the second run, no completely clear portion being observed below the meniscus.

In the 5th run the rotor was operated at 650 r.p.s. for 40 min. Ten lb./sq. in. was applied to the brake until the speed had fallen to 400 r.p.s., after which the pressure was reduced to 5 lb./sq. in. until the speed had dropped to 100 r.p.s. The brake was then turned off and the rotor allowed to coast freely to a stop. The total time during which the brake was applied was about 35 min., and the time of coasting was about 2 hr., 15 min.

The results were practically identical with those obtained in the 1st and 3rd runs, a completely clear portion appearing at the top of the liquid column.

From these experiments, it seems that even slight application of the brake

at low speeds will cause stirring of the smaller particles whose upper boundary has moved only a short distance down the tubes. However, even strong braking does not appear to disturb them while the speed is high.

The larger particles that have been thrown out of suspension or concentrated below a sharp boundary near the bottom of the tubes do not appear to be disturbed by strong braking, even at low speeds. This conclusion was borne out by a recent series of brake and no-brake runs on haemocyanin, which at a speed of 850 r.p.s. was thrown completely out of suspension in 2 hr. Here moderate continuous application of the brake appeared to produce results as good as those where no braking was used.

Experiment 7. Antex. L498. 1 cc.=⬭=40 M.U. This preparation was centrifuged at 650 r.p.s. (100,000 g.) for 6 hr. Uniform deceleration by means of the air brake brought the centrifuge to rest in 25 min. The contents of each

TABLE 3. RESPONSE OF THYROIDS IN DAY-OLD CHICKS

Centrifuged fractions	1 (top)	2	3	4	5 (bottom)	Control (no injection)
Average weight of thyroids (5 animals)	4.64	4.84	4.4	4.36	3.94	3.43

tube were divided into 3 equal fractions (top, middle and bottom), and similar fractions pooled. Each of the 3 fractions was tested on 3 groups of 21-day-old mice which received total doses of 12, 30, 60 M.U. respectively. The data, shown graphically in figure 1, V, show that no concentration occurred.

Experiment 8. Antex. L503. 1 cc.=⬭=5 M.U. This preparation was divided into 2 portions. The 1st was centrifuged at 650 r.p.s. for 6 hr. and the centrifuge allowed to coast to a stop in 4 hr. The 2nd was similarly centrifuged, but the air brake applied at the close of the 6-hr. run stopped the centrifuge in 20 min. The results, which are shown in figure 1, VI, are evidence that braking the centrifuge interferes with the sedimentation of the relatively light molecular substances with which we are concerned. The upper fraction of the unbraked run gave ovaries and uteri no heavier than uninjected controls. The failure of the lower fraction to elicit a still greater response than that recorded is unexpected. It was necessary in this experiment to use a strain of mice from an outside source. Subsequent evidence has shown this strain to be less sensitive and uniform in its response. As mentioned previously, braking does not seem to interfere with the concentration of heavier substances, such as haemocyanin.

Experiment 9. Menopause urine preparation. L521. 1 cc.=⬭=20 mg. dry powder=⬭=50 M.U. Twenty-five cc. (=⬭=500 mg.=⬭=1250 M.U.) of this preparation was centrifuged at 800 r.p.s. for 6 hr. and the centrifuge allowed to coast to a stop (required 4 hr.). The contents of each of 5 tubes was divided into 2 fractions, the upper containing approximately 4.5 cc. and the lower 0.5 cc. The similar fractions were pooled (a portion was unfortunately lost during manipulation), precipitated by acetone, collected and dried. The

resulting dry powders were weighed and assayed. The data, presented in table 2, indicate that the hormone and certain of the impurities are concentrated at a uniform rate. The activity per mg. of solids is of the same order in the centrifuged fractions and in the original uncentrifuged material. However, it is clearly shown by the relative amounts recovered from the 2 fractions that an actual concentration does take place. This indicates a relatively high molecular weight for the hormone.

Experiment 10. Flavianic extract of beef anterior lobe. Thyrotropic hormone. 1 cc.=⌬=20 mg. dry powder. This preparation was centrifuged at 650 r.p.s. for 3 hr. and the centrifuge allowed to coast to a stop in 4 hr. Five fractions of 1 cc. each were made from the contents of each tube, and similar fractions pooled. They were numbered 1 to 5 from top to bottom. The fractions were tested in day-old chicks for thyrotropic potency. The results show that each fraction, with the possible exception of the bottom, gave a significant response but that no concentration of the hormone was obtained (table 3).[5]

The centrifuged flavianate fractions were subsequently acidified with H_2SO_4. A much denser precipitate in fraction 5 indicated that a considerably greater amount of inert protein had been thrown down into the fraction.

Thus, although the centrifuge at these speeds will concentrate the gonadotropic principle from castrate urine or mare serum preparations, it apparently fails to concentrate the thyrotropic flavianic acid preparations. This indicates a considerable difference in the molecular weights of active principles and suggests the possibility that with further experimentation anterior lobe hormones may be further separated and purified by centrifugation.

SUMMARY

The gonadotropic hormones of pregnant mare serum (Antex), of castrate urine and a thyrotropic flavianate extract of beef hypophysis have been subjected to 150,000-200,000 g. in an ultra-centrifuge of improved design for periods of from 4 to 6 hr. A marked concentration of the mare serum and castrate urine preparations was effected, but little if any concentration of the thyrotropic flavianate extract was obtained. In all instances a sludge was thrown down in the base of the tubes in the form of a gelatinous pellet. These pellets were found to be without gonadotropic or thyrotropic potency. Their elimination represented a mechanical purification of the preparations. The centrifuged gonadotropic extracts were assayed by injection into 21-day-old female mice, the weights of ovaries and uteri being the measure of gonad-stimulating potency. The thyrotropic potency was assayed by the weight response of thyroids of injected day-old chicks.

It was demonstrated that braking the centrifuge to accomplish a comparatively rapid deceleration after the desired run interfered with the concentrations of the extracts. The experiments demonstrate the feasibility of concentration and purification of hormone preparations, and suggest the possibility of actual hormone separations by the same means. Such experiments are now in progress.

[5] We wish to thank Dr. George Smelser for his assistance in the assays by the method which he has described.

THE EFFECT OF HYPOPHYSECTOMY ON THE RESTORATION OF THE LIVER FOLLOWING PARTIAL HEPATECTOMY IN RATS

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The intimate relationship between the function of the pituitary gland and general body growth has long since been well established, and the profound effect of hypophysectomy on general body growth is well known. Many attempts have been made also to associate the growth of tumors with the secretory activity of the pituitary gland (1). The remarkable rate of growth exemplified by the restoration of the liver, which occurs after damage to the liver by certain diseases and poisons, and also after partial hepatectomy, has already been the subject of extensive experimental investigations, the historical and histological aspects of which were thoroughly reviewed by Fishback in 1929 (2). A quantitative study of the cell growth that constitutes this restoration of the liver has recently been made by Brues, Drury and Brues in this laboratory (3). In the following experiments we have enlisted the method for partial hepatectomy in rats used by Higgins and Anderson (4), and have measured the restoration of the liver following this procedure in hypophysectomized rats in order to examine the influence of the growth hormone of the pituitary gland on the growth of liver tissue.

The ability of the liver to restore itself after partial hepatectomy is extraordinary in the rapidity of its occurrence. When based on the degree of restoration found in normal rats at the end of the 3rd postoperative day, the increase in the weight of the liver remnant occurs at the rate of 0.42 per cent of the body weight for each 24 hours during this time. In a man weighing 75 kg. this would equal 315 gm., and be comparable to the production of a mass of tissue equal in size to both his kidneys or to about one-fifth the normal size of his liver each day for the first 3 days. Davis, Hall and Whipple (5) have made a similar comparison of this growth and state: "If this speed of growth should be attained by a malignant tumor it would give most astounding clinical histories—medullary cancers of the breast could grow to huge size within a week or less, etc." In this study we have attempted experimentally to measure the influence of the growth hormone of the pituitary gland on this extraordinary example of growth by examining the rate of restoration of the liver after hypophysectomy.

Fishback (6) has pointed out an objection to the use of the term regeneration to designate the type of growth here represented, namely, that the restoration occurs within the lobules remaining after operation, and not

at the pedicle at the site of removal of the liver mass. Therefore, it does not fall within the biological definition of 'regeneration,' which demands that the growth take place at the site of removal. This is an objection to the term alone, however, and not to the experimental method as applied to the problem at hand.

Whether the stimulus to the restoration which occurs after partial surgical removal is a functional one arising from a physiological lack (7), or a simple stimulus to restoration of the portal bed (8), is immaterial to the present investigation, which was intended primarily to measure the rate and extent of increase in the mass of cells resulting after partial hepatectomy. It is, in any event, growth in its broadest sense, since Brues, Drury and Brues (3) have demonstrated an increase in the actual number of liver cells as well as an increase in liver mass following this procedure.

EXPERIMENTAL METHOD

Hypophysectomy was performed on a series of albino rats from the pure inbred Slonaker strain of our laboratory when they were between 55 and 91 days old, and weighed between 59 and 143 gm. In order to permit continued retraction of the pharynx during the operation, a tracheotomy, consisting only of a small transverse slit between two tracheal cartilages, was performed on all rats as a part of the operative procedure. No tracheal cannula was used, and a small pledget of cotton moistened with ether and laid over the tracheotomy opening was sufficient for anesthesia during the remainder of the operation. The base of the skull was then exposed by the parapharyngeal route, and the pituitary was reached by drilling a hole in the base of the skull with a dental drill. Using a Beebe loupe mounted with a small spectacle light, the gland was removed under direct vision with the aid of a small glass suction tube. The loupe gave a magnification of 2½ times, and was found to be more satisfactory than the more cumbersome and expensive types of dissecting microscopes commonly employed in this procedure. The operation was often completed in less than 5 minutes, and gave a primary mortality of between 5 and 10 per cent. The number of animals that later died of intercurrent infections was very small. By this operation the entire pituitary gland, together with a portion of the stalk, was removed. It is possible that portions of the stalk remain after hypophysectomy performed in this manner, but this does not affect the development of the usual post-hypophysectomy syndrome as described by Smith (9). This syndrome quickly developed in all our animals, and was clearly recognizable at the end of about two weeks. At the conclusion of the operation, a small piece of muscle was left in the pituitary fossa to arrest hemorrhage, and a small portion of the skin incision was left unsutured to prevent the development of postoperative subcutaneous emphysema from air escaping through the slit in the trachea. The wounds usually closed on the 2nd day and healed without incident.

After hypophysectomy the animals were placed again on their usual diet of Dog Chow biscuits, skimmed milk, and occasional greens. After 3 to 10 weeks had elapsed, a partial hepatectomy was performed under ether anesthesia in

both hypophysectomized and control groups. A few animals that had been hypophysectomized 6 to 7 months previously showed no demonstrable difference in results from those obtained in animals hypophysectomized only 3 weeks previously. The animals were fasted preoperatively for 3 to 5 hours. At operation the bifurcate median and left lateral lobes, hereafter called the main liver mass, were removed, leaving the irregularly-shaped posterior lobe, hereafter called the posterior lobe. This procedure removed approximately two-thirds of the liver tissue. A coarse ligature of linen was placed about the pedicle of the main liver mass as near to the hilum of the liver as was possible

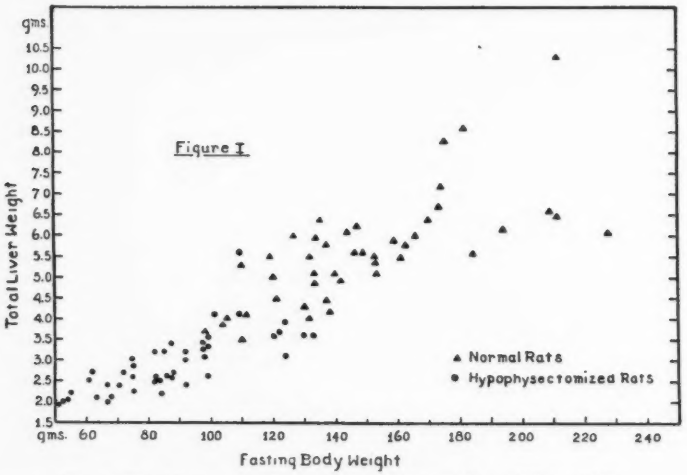

Fig. 1. Showing poor correlation between body weight and liver weight.

without constricting the hilar vessels of the remaining posterior lobe. The main liver mass was then carefully cut off and gently squeezed to permit its free blood to be blotted away. Its weight was determined to the nearest milligram. The abdomen was closed with two layers of linen sutures. The same procedure was repeated upon another series of rats which had not been hypophysectomized.

Both groups were placed on a bread and skimmed milk diet after operation, since Davis and Whipple (10) have shown this to give optimum repair after liver injury. Brues, Drury and Brues (3) have shown that this diet also gives optimum mass and cell restoration after partial hepatectomy. Without exception, the wounds in the abdominal wall healed by first intention, in the hypophysectomized animals as well as in those not hypophysectomized. The normal animals withstood the procedure very well, but the hypophysectomized animals suffered a mortality varying between 20 and 40 per cent for various series, especially among animals which had been hypophysectomized several weeks earlier and were somewhat emaciated. The hypophysectomized animals tolerated only a very light ether anesthesia during the operation.

After periods varying in length from 3 to 16 days after partial hepatec-

tomy the control and hypophysectomized rats were both fasted again, and then killed by decapitation. The regenerating posterior lobe was carefully removed, gently squeezed, blotted free of blood, and weighed. The region of the pituitary was carefully examined with a binocular magnifying loupe for glandular remnants. Since the syndrome which develops after hypophysectomy is so clearly recognizable, and fails to appear if any glandular tissue remains, histological examination by serial section was not considered necessary.

METHOD OF CALCULATION

Previous investigators in this field, with the exception of Brues, Drury and Brues (3), have calculated the degree of liver restoration on the basis of body weight. The latter investigators have pointed out the large error in this method of calculation. Among our own animals the difference in the weights of the liver in fasting rats of equal body weight was sometimes found to be as great as 60 per cent; and rats differing in body weight by as much as 30 gm. not infrequently had equal liver weights. Moreover, it was assumed that the same ratio of liver weight to body weight could not be applied to both hypophysectomized and normal rats, since Smith (9) had reported that the loss of weight suffered by the liver after hypophysectomy was relatively greater than the loss in general body weight. Lee and Ayres (11) reported also that the liver weights in hypophysectomized rats were relatively lower than those of normal rats in paired feeding experiments.

In a preliminary series of 44 normal and 44 hypophysectomized rats, which had fasted for from 3 to 14 hours, and were killed for use in other experiments in our laboratory, the liver was carefully removed and blotted free of blood immediately upon death, the main liver mass removed as at operation, and the main liver mass and posterior lobe weighed separately. Of these animals, 36 had been hypophysectomized for 23 to 53 days (the majority of these between 34 and 53 days); and the remaining 8 rats had been hypophysectomized for 105 to 123 days. The majority of the hypophysectomized rats had weighed between 75 and 130 gm. at the time of hypophysectomy.

Distribution charts were constructed to test the correlation between the total liver weight and the body weight, and also between the total liver weight and the weight of the main liver mass. The superiority of the weight of the main liver mass over body weight as a basis for the calculation of the total liver weight was clearly shown (fig. 1, 2). The proportion of the liver made up by the main liver mass and by the posterior lobe was extraordinarily constant. This was true even at extremes of total liver weights and body weights. The total liver weight was found to be 3.80 per cent ($\sigma = \pm 0.57$; $\sigma/m = 0.150$) of the body weight in the normal rats and 3.42 per cent ($\sigma = \pm 0.54$; $\sigma/m = 0.158$) in the hypophysectomized rats; whereas the main liver mass was found to be 66.83 per cent ($\sigma = \pm 3.54$; $\sigma/m = 0.053$) of the total liver weight among the normal rats and 66.76 per cent ($\sigma = \pm 2.21$; $\sigma/m = 0.033$) among the hypophysectomized rats. This larger series confirms the findings of Brues, Drury and Brues (3) that the relationship of

the weight of the entire liver to the weight of the main liver mass is several times as consistent as the relationship of the weight of the entire liver to body weight.

Since the relationship of the main liver mass to total liver weight is so constant, we have calculated the weight of the remaining posterior lobe

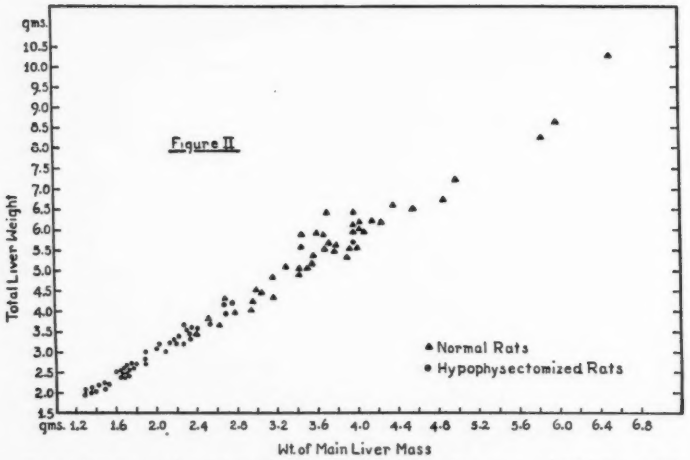

Fig. 2. Showing good correlation between weight of main liver mass and total liver weight.

(PL), and also the total liver weight (TL), from the weight of the main liver mass (MLM) removed at operation by using the following formulae:

$$TL = \frac{MLM}{0.668} \quad \text{or} \quad 1.497\ MLM$$

$$\text{and} \quad PL = 33.2\% \text{ of } TL = \frac{MLM}{2.01} \quad \text{or} \quad 0.497\ MLM$$

The percentage of restoration may be calculated at the end of any desired period by comparing the weight of the regenerating posterior lobe (RPL) with the calculated total original liver weight (TL) as follows:

$$\text{percentage of restoration} = \frac{RPL \times 100}{TL} = \frac{RPL \times 100}{MLM/0.668} = \frac{66.8\ RPL}{MLM}$$

The average ratio (0.668) of main liver mass to total liver was used in the calculations for both the normal and hypophysectomized groups, since the ratios were found to be practically identical, *i.e.*, 0.6683 and 0.6676 respectively.

The degree of restoration of the liver was found to be most clearly visualized if the weight of the tissue found at death were expressed in percentage of the calculated original liver weight. At the completion of the

operation of partial hepatectomy as described above, 33.2 per cent of the original liver weight remained in the posterior lobe. This has been indicated in the first group of columns in figure 3 to facilitate comparison with the results charted in the succeeding columns of the figure.

RESULTS

In the first series of experiments it appeared that the control rats restored their livers at a greater rate than the hypophysectomized rats. It was suspected, however, that this difference might be accounted for by the fact that the

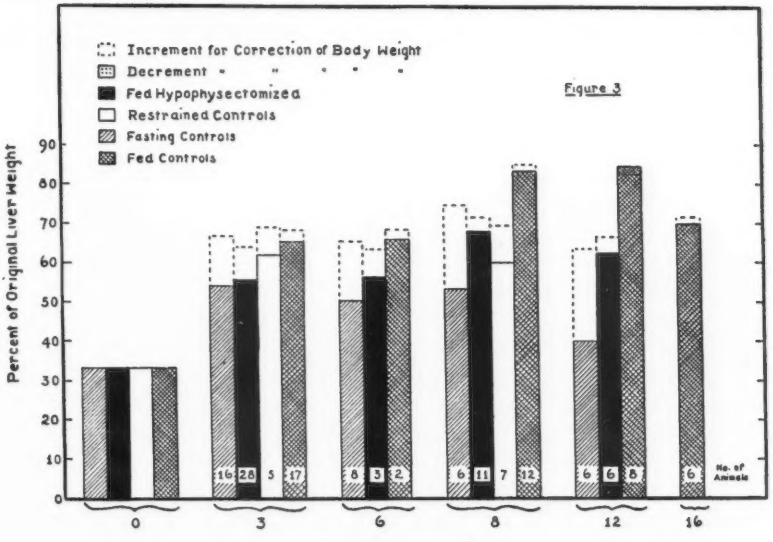

Fig. 3. Liver regeneration after partial hepatectomy.

hypophysectomized rats ate just enough food to sustain life after operation, whereas the appetite of the control rats remained good.

After hypophysectomy the appetite was always extremely poor, and in a comparatively short time the body stores of fat were exhausted. A comparison of the regenerative power of normal and hypophysectomized animals under fasting conditions, to eliminate this difference in food consumption, could not be made since, in spite of numerous trials, no hypophysectomized animal survived a fasting period of more than 36 hours. Rats that had been hypophysectomized for several weeks often failed to survive 12 hours of complete fasting even without hepatectomy. They often died in what appeared to be convulsions of hypoglycemia, suggesting that the glycogen stores were very low from their self-enforced near-starvation. That the glycogen storage was poor was found experimentally by Phillips and Robb(12) in experiments carried out in this laboratory.

Normal rats, however, were invariably able to survive partial hepatectomy and subsequent fasting for several days. In the accompanying chart, only

those animals are recorded which survived the indicated period and were then killed, since in only these animals could the liver be blotted free of blood and properly weighed immediately after death.

At the end of the 3rd day, fasting normal rats had restored their livers to approximately the same degree as the hypophysectomized rats who had had access to food, but the fasting animals failed to continue to restore their liver tissue beyond this time; and in a few very well-nourished rats which survived 12 days of fasting after partial hepatectomy the liver weight of the posterior lobe had fallen by the time of death almost to its original weight calculated to have been present at the time of operation (fig. 3).

In an attempt to control the difference in food intake between the normal and hypophysectomized rats a 3rd series of experiments was performed, in which control rats were paired with rats to be hypophysectomized. At the beginning of the experiment the weights of the two partners differed in no case by more than 2.5 gm. After hypophysectomy of one member of each of the pairs, the weight of the partner was restrained by withholding all food except an amount just sufficient to maintain its weight at or below the level of its hypophysectomized partner. The hypophysectomized rats showed some loss of weight at first, especially in the case of mature rats with large fat stores. Following this initial fall, their weights became quite stationary, and the restrained partners required very little food to maintain a similar weight. At the time they were killed the restrained rats were in 5 instances lighter in weight than their hypophysectomized partners (3.5, 7.5, 11, 13.5, and 21.5 gm., respectively), and in 2 instances 4.5 gm. heavier. After 3 weeks had elapsed, both members of the pairs were subjected to partial hepatectomy. The hypophysectomized rats in this series restored their livers at approximately the same rate as the earlier series of hypophysectomized animals, and the results of these two hypophysectomized groups have therefore been combined in figure 3.

The restrained rats had restored their livers slightly better than their hypophysectomized partners at the end of 3 days, but slightly less well than fed normal rats. At the end of 8 postoperative days, however, the restrained rats appeared to have failed to restore their weights further. The differences were not great, and the series was too small to be conclusive. At autopsy, a few of the restrained rats gave evidence of having stored a small amount of fat in spite of their greatly restricted food supply, whereas the hypophysectomized rats, even with a slightly greater body weight, showed no similar reserve.

In an effort further to resolve the regeneration of the liver in hypophysectomized animals, we have calculated the number of cells and the weight of liver substance present in the regenerating liver of the hypophysectomized rats in this series after partial hepatectomy. These figures have been compared with those previously reported by Brues, Drury and Brues (3) for normal rats of the same strain which had been subjected to the same procedure. The results are shown in table 1, and show that the retardation in the hypophysectomized animals, as compared with the normals, was of approxi-

mately the same magnitude with reference to the number of cells and weight of tissue. In both instances the values for the hypophysectomized animals ranged approximately between two-thirds and three-fourths of those for normal animals. This may be interpreted as meaning that both aspects of growth were similarly affected: namely, the increase in substance of the cells as represented by the weight of the regenerating tissue, and the multiplication of the cells as represented by the number of cells.

The number of cells undergoing mitotic division in the regenerating livers of the hypophysectomized rats were also counted for comparison with the

TABLE 1. COMPARISON OF NUMBER OF CELLS AND WEIGHT OF REGENERATING PORTION OF LIVER IN NORMAL AND HYPOPHYSECTOMIZED ANIMALS

Days after Oper.	No. of cells in regenerating liver[1]				Ratio Hypophysectomized: Normals	Weight of residual liver[2]				Ratio Hypophysectomized: Normals
	Normal		Hypophysectomized			Normal		Hypophysectomized		
	No. of Rats	%	No. of Rats	%		No. of Rats	%	No. of Rats	%	
0	13	31.6	44	33.2[3]	1.05	13	31.6	44	33.2	1.05
3	7	65.5	13	48.6	0.74	7	79.1	28	55.7	0.70
6	4	78.9	1	47.1	0.60	4	81.0	3	56.4	0.70
8	4	89.0	2	62.3	0.70	4	91.3	11	68.1	0.75
12	4	94.0	3	64.1	0.68	4	85.5	6	62.4	0.73

[1] Expressed as percentage of the number of cells in the entire liver before partial hepatectomy.
[2] Expressed as percentage of the weight of the entire liver, before partial hepatectomy.
[3] Estimated on the basis of comparative weight of the 2 liver portions on the assumption that the average cell size is uniform throughout the liver, as is the case in the normals. The normals in this table have been taken from the series of Brues, Drury, and Brues (3).

number of mitoses found in the regenerating livers of normal rats previously reported by Brues and Marble (13). The latter workers have reported that only an occasional mitosis (never more than one in 10,000 to 20,000 cells) can be seen in the resting liver. In regenerating livers of hypophysectomized rats at the end of the 3rd day after operation, an average of 0.56 per cent of cells in mitosis were found in hypophysectomized animals, as compared with an average of 0.63 per cent of cells in mitosis found in regenerating livers of normal animals at the end of the same period, previously reported by Brues and Marble (13).

DISCUSSION

Higgins and Anderson (4), and also Brues, Drury and Brues (3), have shown that liver restoration after partial hepatectomy is somewhat cyclic, and may arbitrarily be divided into 3 periods. The first period roughly covers the first 3 days, during which the increase in mass is rapid. Brues, Drury and Brues suggest that the first increment probably consists chiefly of fat. The second period, including approximately the 3rd to the 6th days, is one of lag in rate of restoration. In the third period, from the 6th to the 9th days, growth is again accelerated, conditioned perhaps as Brues, Drury and Brues suggest, by the fact that the animals are gaining weight rapidly during this period.

The difference in restorative power between normal and hypophysectomized rats is less when measured at the end of the first 3-day period than

subsequently. This suggests that, whatever the stimulus may be to the first increase, it is not greatly different in the hypophysectomized and in the normal rats, or even in fasting rats. During the time when the second period of accelerated growth occurs in the normal rats, the difference between the hypophysectomized and normal rats becomes greater. It is during this phase that the greatest differences are observed between fed and fasting animals also, suggesting that differences in intake of food have the most profound effect during this period. If correction be made for loss or gain in body weight after partial hepatectomy, the differences in degree of restoration in the groups of normal rats at 8 and 12 days is emphasized. This is shown in figure 3 by the superadded columns which show the correction for change in body weight.

From our data (fig. 3), it will be observed that the hypophysectomized rats, when killed at the end of 6 days, show a suggestive lag in rate of restoration when compared with the rate prior to this time and subsequent to it. This lag has previously been observed in normal and fasting rats at the end of this period by Higgins and Anderson (4) and also by Brues, Drury and Brues (3).

Smith, Greenwood and Foster (14) have shown that the metabolism of hypophysectomized rats is lower than that of normal rats. There may thus be a lessened functional need for liver tissue in the former. It is remarkable that the differences in rate or degree of restoration of the liver between normal and hypophysectomized rats are not greater in view of a completely deranged body economy in the latter, with complete arrest of body growth and of sexual development, anorexia, lower body metabolism, and poorer glycogen storage. In contrast with the ability of the hypophysectomized rats to restore their liver, Perla (15) has recently reported that hypophysectomy completely inhibits the regeneration of splenic tissue after partial splenectomy.

The lessened capacity of hypophysectomized rats to restore their livers after partial surgical removal appears to be related chiefly to differences in alimentation due to loss of appetite on the part of the hypophysectomized rats. The difference may possibly also be accentuated by poorer assimilation of food in the hypophysectomized rats, as suggested by the poorer utilization of carbohydrate and storage of glycogen shown by Phillips and Robb (12). The fact that Davis and Whipple (10), and also Brues, Drury and Brues (3) have shown that diet markedly influences liver restoration also supports the suggestion that alimentation is probably a very important factor in the difference between the two groups. The difference in restorative ability between normal and hypophysectomized rats in our data is not great, however, and although it is suggestive it does not convincingly demonstrate a specific primary effect related to the absence of the growth-promoting hormone of the pituitary gland.

SUMMARY

The effect of hypophysectomy upon the ability of rats to restore their liver tissue after partial hepatectomy has been examined.

Distribution charts have been constructed from observations in a control series of 44 normal and 44 hypophysectomized rats to show the superiority of using the weight of the main liver mass removed at operation, instead of

body weight, as the basis for the calculation of the amount of liver tissue remaining after partial hepatectomy. The degree of restoration may then easily be calculated with considerable accuracy as the percentage of calculated original total liver weight. Using this basis of calculation, restoration of liver tissue after partial surgical removal occurred in hypophysectomized rats at a somewhat slower rate than in normal rats on an optimum diet, but at a more rapid rate than in fasting rats, especially beyond the 3rd postoperative day.

Rats whose body weights had been restrained to or below the level of their hypophysectomized partners restored their livers slightly more rapidly than hypophysectomized rats during the first 3 postoperative days, but more slowly than normal unrestrained rats. By the 8th postoperative day, the hypophysectomized rats appeared to have exceeded the restrained rats in percentage of liver restored, but the number of restrained animals was too small to be conclusive.

Calculation of the number of cells and the weight of liver substance present in the regenerating portion demonstrated that in both respects the values for the hypophysectomized animals ranged approximately between two-thirds and three-fourths of those for normal animals. This may be interpreted as showing that the mass and multiplication of the cells were similarly affected. The percentage of cells undergoing mitosis in the regenerating liver was found to be 0.56 in the hypophysectomized animals and 0.63 in normal animals at the end of the 3rd postoperative day.

Although there appears to be a slightly lessened ability to restore liver substance after partial hepatectomy among the hypophysectomized animals, as compared with animals not hypophysectomized, this may largely be attributed to differences in amount of food ingested by the two groups. The difference is too small to conclude that a direct effect from the absence of the growth-promoting hormone of the pituitary gland is shown upon this example of growth in a parenchymatous organ.

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ON THE RELIABILITY OF PRESENT METHODS FOR CHARACTERIZING TWO GONADOTROPIC HORMONES, FOLLICLE-STIMULATOR AND LUTEINIZER

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The view that the physiological response to extracts having gonadotropic activity is dependent upon the relative proportion of two gonadotropic hormones, follicle-stimulator and luteinizer, has gained fairly wide acceptance. Zondek (1) in an early paper formulated this view. Fevold, Hisaw and Leonard (2) were the first to submit good evidence for the separation of two gonadotropic hormones from the anterior pituitary.

Before taking up the data of the present investigations, it would seem appropriate to enumerate the characteristics attributed to follicle-stimulating hormone (FSH) and luteinizing hormone (LH). As is natural in dealing with preparations of varying degrees of purity, there has been some shifting of opinion regarding the characteristics of the two hormones, and we shall attempt to give the most recent viewpoints.

The following are some means of distinguishing the follicle-stimulator, FSH, from the luteinizer, LH. *A*). In the hypophysectomized immature female rat, LH by itself has no effect. FSH produces pure follicular growth. If an in vitro combination of the two is injected, the ovarian response is greatly above that to be expected by a simple additive effect, and the ovaries are heavily luteinized (3, 4).

B). Very much the same response holds for the intact immature female rat, with the exception that FSH is more likely to produce some luteinization when given alone, the extent of luteinization presumably depending upon the relative activity of the animal's own pituitary in secreting luteinizer (2, 5, 6, and others).

C). In the mature hypophysectomized rabbit, follicular maturation is produced by subcutaneous injection of FSH, and ovulation occurs when a subsequent intravenous injection of FSH plus a trace of LH is administered (7). Ovulation did not occur when either pure FSH or pure LH was used for this second treatment in a limited number of cases. However, in the juvenile rabbit after preliminary treatment with FSH, both mixtures of FSH plus LH and pure LH or FSH alone produce ovulation (8, 9).

D). In the male, FSH has a gametokinetic effect, whereas LH produces its predominant effect on the interstitial tissue. The in vitro combination of the two has an augmented effect upon the growth of both testicular components (10, 11). In a more recent paper Evans, et al (12) attribute this effect on the interstitial tissue to a new factor, the interstitial cell-stimulating hormone.

As stated above, the response to a given gonadotropic preparation depends upon the relative proportions of these two hormones. Pregnancy urine is considered predominantly LH, whereas some castrate or menopause urines are presumably FSH. The anterior pituitary contains both factors, although it is believed there may

be marked differences in the relative proportions depending upon species, sex, or reproductive state.

In this paper we are submitting data bearing on the reliability of some methods used in distinguishing the two hormones. We have particularly considered the question as to whether LH has unique properties as regards augmentation and luteinization in the immature female rat which would clearly distinguish it from other substances known to augment gonadotropic fractions of the pituitary. More specifically the following experiments were conducted.

a), Comparison of the augmenting action of pituitary LH, zinc sulfate, casein and egg albumin when mixed in vitro with a pituitary gonadotropic fraction, pituitary synergist, prepared according to the method of Evans, et al (12). *b),* Studies on the augmenting action of LH on pituitary synergist when the two substances were given simultaneously but at different sites. *c),* Studies on the augmenting action of LH on pituitary synergist when the two substances were injected on successive days. *d),* Studies on the effect of various augmenting agents on pituitary FSH prepared according to the method of Fevold, et al (5, 6). *e),* Comparison of the relative activity of FSH and LH extracts in the mature rabbit and immature rat. *f),* Attempts to augment mare gonadotropic hormone. *g),* Studies on the effects of various extracts when injected into 18-day-old rats.

TABLE 1. COMPARISON OF THE EFFECTIVENESS OF PROTEINS AND LH IN AUGMENTING THE EFFECT OF SYNERGIST[1]

Material combined with synergist	No. of rats with estrous smears	No. of rats with corpora	Average No. of corpora	Average ovarian weights Mg.	*P* values synergist vs. combination (ovarian weights)
None	2	12	7.5± .74	33.0	—
B78 (LH)[2]	none	12	21.3±1.5	44.3	$0.01>P$
Egg albumin	7	12	19.4±2.8	42.6	$0.05>P>0.02$
Casein	11	10	15 ±2.7	38.2	$0.2>P>0.1$

[1] Litter-mate sisters were scattered among the various groups, 12 rats in each group. All rats received a total of 0.5 mg. *B108* plus 10 mg. of the augmenting substance.
[2] This preparation had a slight gonadotropic activity when administered alone, producing corpora in the ovaries of 1 of a group of 12 rats injected at this dose level.

EXPERIMENTAL METHODS

Augmentation of pituitary synergist. The augmentation reaction was one of the first points presented as evidence that the gonadotropic response to pituitary extracts was the result of the dual action of two gonadotropic substances (2). Several non-specific substances have been shown to augment the pituitary gonadotropic fraction known as synergist. Maxwell (13) augmented it with zinc sulfate. We (14) showed that it could be augmented by egg albumin and casein. Evans, et al (3) referred in a footnote to a similar augmentation. We felt that this augmenting action of proteins was of considerable importance because this meant that any augmentation obtained with a

given extract might be referred to inert protein present, unless it could be shown that the qualitative or quantitative response differed in some respect from that obtained with protein. It seems clear, in fact, that the augmentation which we (15) obtained by combining an inert-by-itself mare serum fraction with pituitary synergist was due to the serum protein content of the fraction. Thus we now believe that we referred erroneously, or at least ambiguously, to two gonadotropic substances in mare serum. Furthermore, it appears that the augmenting effect of sera of non-pregnant animals upon pituitary synergist

TABLE 2. COMPARISON OF MINIMAL AMOUNTS OF VARIOUS SUBSTANCES REQUIRED TO AUGMENT 0.5 MG. OF SYNERGIST (*B108*)

Material combined with 0.5 mg. synergist	Dose Mg.	No. of rats	No. of rats with estrous smears	No. of rats with corpora	Average ovarian weights Mg.	*P* values ovarian weights of synergist vs. combination
None		48	8	44	30.9	—
Zinc sulfate	20	3	3	3	70.7	$0.01>P$
	10	6	6	5	70.7	$0.01>P$
	5	3	3	3	60.7	$0.01>P$
	2.5	6	6	6	63.0	$0.01>P$
	1.25	6	6	6	55.5	$0.01>P$
	.6	6	4	6	38.0	$0.2>P>0.1$
	.3	5	1	3	29.4	
Casein	10	18	14	16	35.8	$0.05>P>0.02$
	5	3	1	3	46.7	$0.01>P$
	2.5	6	4	6	35.7	$0.2>P>0.1$
	1.25	5	2	5	30.2	
B63, pituitary LH	20	3	none	3	38.7	$0.2>P>0.1$
	10	6	1	6	41.0	$0.01>P$
	5	3	none	1	24.7	

reported by Evans, Simpson, and Auston (16) can be similarly explained without assuming the presence of a 'prolan-like' substance.

For these reasons we were anxious to compare the effects of pituitary LH with other aumenting agents. In testing for augmentation, the following procedure was used. The synergist and the augmenting material were weighed and taken up in such a volume of sterile saline solution that the daily dose per rat was 2 cc. These solutions were made up on the morning of the first injection and stored in the refrigerator at 34°F. between injections. Subcutaneous injections were made once daily into immature female rats on the 25th, 26th, and 27th days, with necropsy on the 30th day of age. The same synergist preparation was used throughout the first 4 sections of this paper. We had previously shown (14) that egg albumin and casein augment the response to synergist under these conditions. We now wished to compare the augmentation produced by these proteins with that produced by pituitary LH. Table 1 gives the result of this test. We see no marked differences in the augmentation produced by these various substances. Furthermore, all of these substances increased the number of corpora 2 to 3 times. The principal difference noted was in the character of the vaginal smear. We have repeatedly found that LH fractions inhibit the occurrence of estrous smears in rats of this

age, but not in rats injected on the 18th day. On the other hand, casein and egg albumin in general increase the incidence. Recently Nathanson, Fevold and Jennison (17) found a similar inhibition by postpartum urine and commercial prolactin, and believe this is due to the LH these substances contain.

Thus it appears that LH cannot be considered simply as an inert protein. As a matter of fact, all of our LH fractions do have a slight gonadotropic activity of their own, occasionally producing corpora in the ovaries of immature rats without causing any appreciable increase in the ovarian weight. Since our synergist when injected alone produced corpora, one might suggest that our failure to obtain greater augmentation by LH was due to the fact that our synergist already contained optimal amounts of LH. We have met this objection later in this paper by the use of follicle stimulators prepared according to the method of Fevold, et al (5, 6). This first experiment, then does not disclose any significant difference between LH and protein as regards the ovarian response.

Minimum amount to produce augmentation. We next combined a constant amount of synergist with varying amounts of LH, zinc sulfate or casein (table 2). Zinc sulfate was far more effective than any other augmenting agent, producing significant augmentation when a total dose of 1.25 mg. was used. Casein appears to be slightly more efficient than LH, but this difference is probably not significant. At any rate this series of tests did not give any indication of a specific augmenting action of LH. Fevold and Hisaw (6) reported augmentation of FSH with as little as 0.02 mg. LH, and they did not obtain maximum augmentation until 2 mg. of LH was given. This is in contrast to our results in which increased augmentation is obtained over a narrow range of LH increases.

It is interesting to note that in no case was this synergist, *B108*, augmented as markedly as a previous synergist preparation, A 1 (15). It would have been desirable to run further tests on this latter preparation but unfortunately the supply was exhausted. We have since made several synergist preparations which we could not augment by any of these substances including two pituitary LH preparations. We are at a loss to account for these differences, but we have encountered them several times with various preparations including purified FSH.

Separate injection experiments. Maxwell (13) proposed that the augmentation produced by zinc sulfate was due to its action in delaying the absorption of the active material. In order for the augmenting agent to act in this manner it must be combined with the synergist in vitro. However, if LH produces its augmentation by virtue of its existence as a separate hormone, it should be just as effective when the synergist and LH are injected separately at different sites as when the two are combined. To test this point we injected a group of rats with a constant amount (0.5 mg. total) of synergist in a small volume (1 cc. daily) well up in the left shoulder region. We then injected saline, zinc sulfate or LH, in a volume of 1 cc. daily, on the right side, taking care to prevent subcutaneous mixing of the two solutions. These injections were repeated daily for 3 days, with necropsy 5 days after the first injection. The

ovarian weights of 48 rats receiving only synergist averaged 30.9 mg. For 18 rats receiving synergist and zinc sulfate the ovaries averaged 38.2 mg., whereas for 5 rats receiving synergist and a pituitary LH preparation this average was 28.6 mg. Zinc sulfate thus appears to have caused a slight augmentation, whereas LH did not. This augmenting action of zinc sulfate may have been due to a slight subcutaneous mixing of the injected fluids, or to a rapid absorption of both materials into the blood stream, and their combination there to decrease the rate of excretion of the active material. Since augmentation was obtained when these substances were mixed in vitro but not when they were injected separately, it would appear that LH, like other augmenting agents, must be physically or chemically bound to the synergist in order to be effective.

Successive injection experiments. Several authors have suggested that FSH and LH act in sequence, and best results are obtained when the two are injected in the above order. Fevold, et al (2) observed augmentation when they injected gonad-stimulating hormone, FSH, followed by LH. However, their controls are inadequate, since they give no figures for gonad-stimulating hormone alone or LH alone when injected under the same conditions. Neither did they report the results of any experiment where the order of the injections was reversed. In other words, the results of their two experiments are not comparable since in one case the gonad-stimulating hormone was administered in 5 daily injections whereas in the other case the same total amount was given in two daily injections. Wallen-Lawrence (18) has reported similar experiments with adequate controls of all types. She obtained greater augmentation when she administered FSH followed by LH than when the order of the injections was reversed, and considered this proof of the existence of two separate gonadotropic hormones. However, she overlooked the possibility of another explanation which seems more logical when we recall that, in the doses used, her LH produced a considerably greater increase in ovarian weight than did her FSH, and both of these fractions produced a greater effect at 72 hr. than they did at 120 hr. after the first injection. This time relationship is very important. Considering this, we should expect a greater response when necropsy is performed at 72 hr. after the injection of the more active material than when the more active material is injected first and necropsy is performed 72 hr. after the injection of the less active material. According to this idea, the greater augmentation obtained when FSH preceded LH was due to a quantitative difference in the amount of gonadotropic hormone in the two preparations, and does not prove the existence of two separate hormones.

We performed some successive injection experiments, using synergist for the first two injections and pituitary LH, zinc sulfate or egg albumin for the next two with necropsy 5 days after the beginning of the treatment. Our LH had practically no effect on the ovary when administered alone in this dose. The following average ovarian weights were obtained: synergist alone, 32.2 mg. (17 rats); synergist and zinc sulfate, 35.2 mg. (10 rats); synergist and pituitary LH, 36.2 mg. (10 rats); synergist and egg albumin, 33.7 mg. (6 rats). In no case was any significant augmentation obtained.

From the four types of experiments herein discussed, no evidence could be obtained in support of the view that LH acts as a specific augmenting agent,

but rather the view is taken that the augmenting action of this fracion is due to its protein content plus the gonadotropic activity that this fraction contains.

Studies on pituitary FSH. Among the earliest to present good evidence for the separation of two gonadotropic fractions from pituitary were Fevold, Hisaw and associates (2, 5, 6). More recently, Wallen-Lawrence (18) and Evans, et al (3, 19, 20) have devised methods of bringing about a separation of effects, although these authors were unable to repeat successfully the work

TABLE 3. RESPONSE OF THE IMMATURE RAT TO INJECTIONS OF PITUITARY FRACTIONS PREPARED ACCORDING TO FEVOLD'S METHOD[1]

No. of rats	Dose of *B152*[2] cc.	Dose of *B153*[3] mg.	Dose of egg albumin mg.	No. with estrous smears	No. with corpora	Average uterine weight mg.	Average ovarian weight mg.
8	0.25	—	—	1	none	25.3	16.6
6	0.5	—	—	4	3	32.5	18.7
6	0.5	0.5	—	none	2	23.7	20.2
6	0.5	5.0	—	none	4	28.5	20.5
6	0.5	—	0.5	3	3	33.3	17.3
6	0.5	—	5.0	5	2	38.5	20.2
6	0.5	—	25.0	6	5	35.0	19.7
6	1.0	—	—	4	3	36.8	24.7
6	1.0	0.5	—	none	3	27.0	22.5
6	1.0	5.0	—	none	2	24.0	20.2
6	1.0	—	0.5	5	3	34.0	23.3
6	1.0	—	5.0	2	1	27.0	15.3
6	1.0	—	25.0	3	3	34.5	29.7
12	2.0	—	—	6	9	35.6	31.5
6	2.0	5.0	—	none	5	24.7	32.5
6	2.0	—	5.0	1	5	27.8	28.5
4	3.0	—	—	none	3	28.8	33.5
6	—	0.5	—	none	1	25.5	15.0
6	—	1.25	—	none	1	25.0	16.7
6	—	2.5	—	none	2	33.8	19.3
8	—	5.0	—	none	4	25.8	19.1

[1] All combinations made *in vitro* and injected in 3 equal doses, starting on the 21st day with necropsy 5 days after the first injection.
[2] *B152*—FSH from pituitary by Fevold's method.
[3] *B153*—LH from pituitary by Fevold's method.

of earlier workers. We have tried these methods on mare serum and sheep pituitary, but have used Fevold's methods for most of this work. We made one preparation from pituitary by Wallen-Lawrence's method. Both fractions obtained produced corpora in ovaries weighing 25 mg. after 3 daily administrations to rats 25 days old, at the beginning of the treatment.

We also made six separate attempts by Fevold's detailed modification of his 1933 and 1934 methods (indirect personal communication). At least 100 gm. of dried sheep pituitary was used in each of these preparations. Preliminary tests were run on the separate FSH fractions, and then the FSH fractions from 3 trials were combined for more extensive testing. When 3 daily injections were made, starting when the rats were 21 days of age, with necropsy 5 days after the first injection, all of these fractions produced corpora in the ovaries of

the test rats. However, when the same dose was administered to 6 rats in 2 injections daily over a period of 4 days, it produced only ripe follicles (ovaries average 49 mg.). A second of our FSH preparations, however, produced corpora in 5 out of 6 rats when injected under these conditions (ovaries average 32 mg.). On the contrary the LH fraction when administered under the same conditions (8 injections) either with or without the addition of FSH did produce corpora at very low levels. These results may indicate that some fractionation of the gonadotropic hormone has occurred, but we are more inclined toward the view that the differences are due merely to physical or chemical changes in the hormone occasioned by the method of preparing the two fractions.

Some other points of interest in the response to one of our FSH fractions (*B152*) and an LH fraction (*B153*), presented in table 3, may be summarized: *a)*, In this case, neither LH nor egg albumin caused any noticeable increase in the ovarian weights over those produced by FSH alone in the doses used; *b)*, Neither of these substances had any significant effect on the number of rats having corpora in their ovaries; *c)*, At low levels of FSH the addition of egg albumin increased the incidence of estrous smears; at higher levels it was without effect; *d)*, The addition of LH to FSH prevented the occurrence of estrous smears in all cases; *e)*, The addition of egg albumin to FSH had no consistent effect on the uterine weights. The addition of LH, however, decreased the uterine weights in all cases.

A word should be said about the corpora lutea produced by the pituitary fractions when administered in 3 injections over a 3-day period. These are usually small structures, and in our experience ovulation is infrequent irrespective of the combinations used. It is likely that ovulation could be produced with optimal amounts by proper timing of the injections.

In an earlier part of this paper we called attention to the fact that some of our snyergist preparations could be augmented by both LH and egg albumin, whereas others could not be augmented by either. Here we have a purified FSH fraction which we were also unable to augment. It is difficult to account for this, but others have encountered the same variability. Thus, although Evans, et al (3) and Fevold, et al (5) indicated that the effects of 'pure' FSH can be augmented by casein and tannic acid, Casida (21) and Hellbaum (22) could not obtain augmentation of their pure FSH by materials that did produce augmentation with unfractionated extracts.

Since the FSH fraction when injected alone often caused estrous smears, whereas the addition of LH to the above prevented this reaction, we tested the effect of the combination of LH and estrin in the immature rat. It was first determined that 0.002 cc. of a crude estrin preparation did not induce estrus in a group of 25-day-old rats, but 0.004 cc. induced estrus in all of a group of 8 rats of the same age. The addition of 5 mg. of *B153* (pituitary LH) to this dose of estrin did not alter the character of the response, all rats being in estrus. A single corpus was found in the ovary of one rat receiving estrin plus LH. None of the others contained either ripe follicles or corpora. The ovarian weights averaged 19.4 mg. for the estrin treated and 19.1 mg. for the estrin plus LH treated group. It seems therefore that the LH fraction has an

inhibitory effect on the production of an estrous smear not by virtue of its power to nullify directly the effect of estrin but because it must either prevent the secretion of estrin or exert its inhibitory action through some secondary mediator.

We have discussed some differences between FSH and LH fractions. Also we have indicated that the response to one of these, FSH, can be altered by varying the manner of injection. An important physical difference in the two fractions concerns their solubility. The FSH is very soluble, forming a clear transparent solution. On the other hand LH is insoluble, forming only a crude

TABLE 4. RELATIVE ACTIVITY OF VARIOUS GONADOTROPIC PREPARATIONS IN RATS AND RABBITS

Material	Amount required to produce a response in half the injected rats[1]	Amount required to cause ovulation in the rabbit[2]	Ratio rat dose:rabbit dose
B152 soluble pituitary FSH	0.5 cc.	1/8 cc.	4:1
B153 insoluble pituitary LH	5 mg.	1/16 mg.	80:1
B154 fraction of *B153* not soluble in dilute alkali	4 mg.	1/16 mg.	64:1
B155 fraction of *B153* soluble in dilute alkali	5 mg.	1/2 mg.	10:1
Mare serum *8*	0.02 cc.	0.32 cc.	0.0625:1

[1] Groups of 4 to 6 rats were used at each level. Subcutaneous injections were used with necropsy 5 days after the first injection.

[2] A total of 34 rabbit tests was made on these fractions. The material was injected intravenously, and the lowest level that produced ovulation in one or more rabbits was chosen as the rabbit dose. In assay, the dose was doubled each time; no intermediate levels were run.

suspension. It is probable therefore that these two fractions are absorbed at different rates. The importance of this will be considered in the next section.

Comparison of relative activity of FSH and LH extracts in the mature rabbit and immature rat. It seems to be still unsettled whether FSH or LH causes ovulation in the mature estrous rabbit. Foster, Foster and Hisaw (7) report that FSH with a trace of LH gives best results. Fevold, Hisaw and Greep (4) caused ovulation by intravenous injection of copper salts, and attribute this to the augmenting action of copper on the gonad-stimulating hormone normally present in the blood, raising this activity to the threshold for ovulation.

We have repeated some of this work and confirm the fact that copper sulfate in doses of 25 mg. will cause ovulation when injected intravenously into isolated mature female rabbits. However, zinc sulfate, casein and egg albumin in doses up to 50 mg. were without effect. In testing pituitary fractions it became evident, first, that the amount required to cause ovulation in the mature rabbit was much less than that causing a response in the immature rat and, second, that there was no constant relationship between the rat and rabbit dose with various fractions. Leonard (36) has reported a similar finding. In our work, the lowest dose which gave any type of a response, ripe follicles, corpora or positive vaginal smears in at least half of the injected rats was chosen to compare with the lowest dose which produced ovulation in one-half, or more, of the injected rabbits. The dose of the test material was doubled each time; no intermediate levels were run. Solubility of the various fractions seemed to play a part here (table 4). The insoluble fractions were consistently relatively more active in the rabbit. This might be explained by the fact that in the rat

subcutaneous injections were used, and therefore the insoluble LH fractions would be much more slowly absorbed from the site of injection than the soluble FSH fractions. Since in the rabbit intravenous injections were used, the rate of absorption plays no part. On the other hand, the very soluble FSH will probably be excreted rapidly in both species, whereas the excretion of insoluble LH will be very slow. Therefore, on the basis of the physical condition of the two fractions, the difference in their effectiveness in the rat and rabbit can be explained by the fact that the soluble fraction is either injected or

TABLE 5. ATTEMPTS TO PRODUCE LUTEINIZATION IN THE OVARIES OF VERY YOUNG RATS[1]

Materials injected	No. of rats	No. with estrous smear	No. with corpora	Average ovarian weight
Single injection				
0.32 cc. serum 17	15	14	None	32.9[2]
0.5 cc. serum 17	4	4	None	51.5
1.0 cc. serum 17	4	4	None	75.8
0.32 cc. serum 17 plus 10 mg. *B78*[3]	4	4	None	27.0
0.32 cc. serum 17 plus 25 mg. *B78*	5	5	None	
1.0 cc. serum 17 plus 25 mg. *B146*[3]	4	4	None	38.3
0.5 cc. *B157*[4]	1	1	1	14
1.0 cc. *B157*	11	7	2	15.6
2.0 cc. *B157*	1	1	None	23
3.0 cc. *B157*	1	1	1	41
1.0 cc. *B157* plus 25 mg. egg albumin	10	5	None	14.7
1.0 cc. *B157* plus 25 mg. *B153*[3]	9	9	None	17.6
1.0 cc. *B157* plus 10 mg. $ZnSO_4$	5	5	1	116.0
25.0 mg. *B153*[5]	6	1	1	15.5
Controls	7	None	None	16.6
Successive injections				
0.32 cc. serum 17 plus 10 mg. *B78* on 21st day	4	4	None	14.8
0.32 cc. serum 17 plus 10 mg. *B63*[3] on 22nd day	4	4	None	37.3
1.0 cc. *B157* plus 25 mg. *B153* on 20th day	5	3	3	21.2

[1] In the single injection experiments, the materials were administered on the 18th day except in one instance noted below. Where combinations were used the materials were mixed *in vitro*. In the successive injection experiments the first injection was made on the 18th day followed by the luteinizer on the 20th to 22nd day. All rats were sacrificed on the 23rd day.
[2] This weight includes oviducts; all others ovaries only.
[3] Pituitary LH.
[4] Pituitary FSH.
[5] In this case the injection was made on the 20th day.

quickly absorbed into the blood stream in both species, and is rapidly excreted so it can act on the end organ, the ovary, for only a short period of time. On the other hand, in the rat the insoluble fraction is only slowly absorbed from the subcutaneous tissue, and therefore never exerts its full action. In the rabbit, however, as it is injected into the blood stream and probably cannot escape by way of the kidney, this insoluble fraction can exert its fullest action, and this activity is continued over a prolonged period of time.

In contrast to pituitary preparations, mare gonadotropic hormone is relatively ineffective in the rabbit as compared to its activity in the rat. Since mare serum is readily absorbed into the blood stream when injected subcutaneously, and is not excreted by the kidney it is free to exert its fullest action in both species. It is logical, therefore, that in this case the rabbit because of its size, should require a larger dose than the rat.

Since the insoluble fractions should be predominantly LH and the soluble fractions FSH, the possibility must be considered that the above difference is attributable to the activity of two different hormones. As stated above, Foster, et al (7) found FSH with a trace of LH to be the most effective agent in producing ovulation in the rabbit. If the fact that our FSH produced corpora in immature rats is evidence that it contains traces of LH, then our FSH should have been more effective than LH in producing ovulation. On the contrary, the reverse was found to be true.

Augmentation of mare gonadotropic hormone. Not all gonadotropic preparations can be augmented. Rather this seems to be a property of pituitary gonadotropic hormone, although agreement on this point is not universal. Thus, although Maxwell (13) obtained marked augmentation of the effects of synergist or crude pituitary preparations by the addition of zinc sulfate, he could not obtain any augmentation of the effects of pregnancy urine by this means. On the other hand, Emery (23) reported augmentation of the effects of antuitrin-S by combining it with zinc sulfate. Likewise, although Hellbaum (24) reported augmentation by recombination of two mare serum fractions, we have not been able to augment any mare serum preparation, regardless of the method tried. One of our purest preparations was active in immature rats in a dose of 0.005 mg., producing corpora in some cases at this level, but could not be augmented by zinc sulfate, casein, egg albumin or mare serum LH. Neither did the addition of zinc sulfate or pituitary LH to untreated mare serum cause any augmentation. Many have considered mare serum as predominantly FSH. If this were true it should be augmented by LH. We have a very extensive series of experiments showing negative results upon augmentation of mare gonadotropic hormone, and are rather skeptical of the undocumented reports that any fraction of it can be augmented.

Attempts to produce luteinization in the ovaries of very young rats. In an earlier paper (25) we showed that mare serum, administered in a single dose to rats 21 to 25 days old, produced corpora in their ovaries when necropsy was performed 5 days after the injection. However, a similar injection in rats 18 days old produced only ripe follicles in this period of time. We have now made further attempts to produce corpora in rats of this age. First of all we found that if the serum was administered in 3 injections on the 18th, 19th, and 20th days of age, corpora were sometimes present 5 days after the first injection. This necessitated the use of single injection experiments, at least as far as the serum was concerned. Some of the results of the experiments with 18-day-old rats are given in table 5. These may be grouped into 4 main categories.

a), Mare serum, either alone or combined with pituitary LH, when administered in 1 injection at 18 days of age, did not produce corpora in the ovaries of any of the test rats, two different LH preparations being used; *b),* When mare serum was injected at 18 days of age and pituitary luteinizer 3 or 4 days later, no corpora were found; *c),* A purified FSH preparation, in a single dose at 18 days of age, produced corpora in the ovaries of 4 out of 14 rats. This was, however, predominantly a follicle-stimulating response, as not more than 2 or 3 corpora were found in any case. The addition of egg albumin, LH or zinc sulfate to the FSH did not increase the number of rats having corpora,

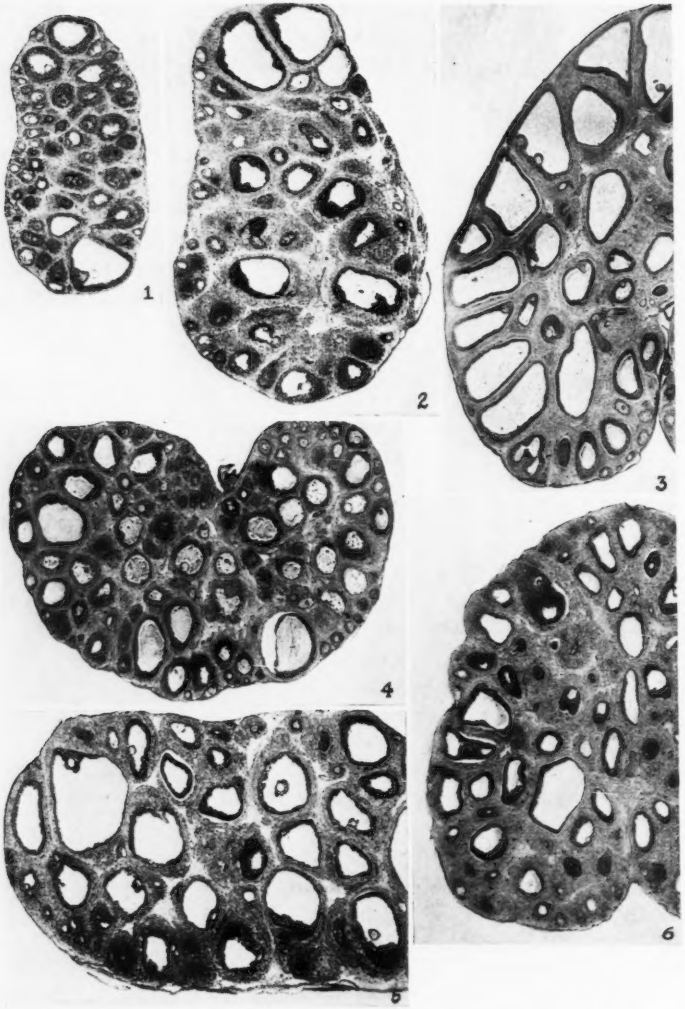

Fig. 1. Ovary of rat *B9921*, uninjected control. Necropsy on the 23rd day of age. Ovarian weight 21 mg. x10.

Fig. 2. Ovary of rat *B9915*, injected with 0.32 cc. of pregnant mare serum on the 18th day of age, necropsy on the 23rd day. Ovarian weight 50 mg. Notice large follicles, absence of corpora lutea and thickening of the theca. x10.

Fig. 3. Ovary of rat *G2627a*, injected with 1 cc. of FSH plus 10 mg. zinc sulfate on the 18th day of age, necropsy on the 23rd. Ovarian weight 103 mg. When zinc is added to FSH it is impossible to distinguish the response in the 18-day-old rat from that of mare serum. (Compare with fig. 5.) x10.

Fig. 4. Ovary of rat *G2625a*, injected with 3 cc. of FSH on the 18th day of age, necropsy on the 23rd. Ovarian weight 41 mg. There are many medium-sized follicles, none of which are as large as in the ovaries of rats receiving mare serum. x10.

Fig. 5. Ovary of rat *G9918*, injected with 10 cc. of pregnant mare serum on the 18th day of age, necropsy on the 23rd. Ovarian weight 142 mg. There are many large follicles but no corpora, although the theca is thickened. x10.

Fig. 6. Ovary of rat *BH3155*, injected with 1 cc. of pregnant mare serum plus 25 mg. pituitary LH in 3 injections on the 18th, 19th and 20th days of age, necropsy on the 23rd. Ovarian weight 70 mg. Compare with fig. 2 and 5. x10.

Fig. 3, 4 and 6 are sections through the greatest diameter of the ovary. Fig. 1, 2 and 5 are at right angles to this plane.

although zinc sulfate caused a marked augmentation in ovarian weight; *d),* When pituitary FSH was injected at 18 days of age followed by LH on the 20th day, corpora were found in the ovaries of 3 out of 5 rats. However, this treatment with LH alone on the 20th day produced corpora in 1 out of 6 cases, so this appears to be merely an additive effect.

Although it has been possible to luteinize the ovaries of these young rats with certain pituitary preparations (synergist also formed corpora), and not with mare serum, there is no evidence that LH is effective in altering the pure follicular response produced by mare serum.

Although there was no conversion of the granulosa into lutein tissues, mare serum did bring about a thecal hypertrophy (fig. 2, 5). The addition of LH to mare serum did not alter the qualitative response (fig. 6). This same thecal hypertrophy was not observed with FSH in amounts producing a similar weight response (fig. 4), but the addition of zinc sulfate to FSH produced a response indistinguishable from mare serum (fig. 3).

DISCUSSION

Two of the main criteria for characterizing FSH and LH are augmentation of ovarian weight and the presence or absence of corpora in the ovaries of injected immature rats. In our experience, casein and egg albumin act very similarly to LH preparations as regards augmentation. We were also unable to find any clear-cut distinction between these substances as regards luteinization when given in conjunction with pituitary synergist or FSH. These findings have led us to a consideration of the adequacy of the theory that the response to any gonadotropic preparation is dependent upon the relative proportion of two gonadotropic hormones, FSH and LH. We shall discuss briefly gonadotropic hormones from several sources.

Serum of pregnant mares. There are reports of the separation of two or more gonadotropic hormones from mare serum, using such relatively simple procedures as fractional precipitation with ammonium sulfate with some control of pH, although apparently no great concentration of the hormone is effected by these methods. On the other hand, a number of highly purified preparations have been made in several laboratories, different procedures being used, yet in each case the final product appears identical to untreated mare serum. Thus, Catchpole and Lyons (26) by acetone ammonia extraction prepared a fraction from mare endometrium which was active in immature rats in a dose of 0.0018 mg. Cartland and Nelson (27) obtained a very pure preparation by fractional precipitation with acetone at carefully controlled pH, active in a dose of 0.00375 mg. By fractional alcoholic precipitation we prepared a fraction active in a dose of 0.005 mg. Gustus, Meyer and Woods (28) obtained some excellent preparations with aluminium hydroxide adsorption. There is also on the market a highly purified Danish preparation, Antex-Leo. None of these reports indicate that these purified preparations have any biological action different from that of untreated mare serum. Mare gonadotropic hormone has been referred to as mainly FSH, although Smith (29) has reported that it produces corpora in hypophysectomized rats.

Pregnancy urine. Here again much the same situation exists. Although

highly purified preparations are available, there are no confirmed reports of the separation of two or more factors. However, as has been shown by a number of authors, the responses to human pregnancy urine and to pregnant mare serum are not identical. Some have referred to pregnancy urine as LH, but actually it is very different from pituitary LH preparations. For example, pregnancy urine causes follicular growth, estrus, and ovulation as well as luteinization in the intact immature rat (unpublished data), whereas pituitary LH preparations when given alone are much more limited in action,

Menopause urine. There is considerable evidence that many menopause urines produce a predominantly follicle-stimulating response.

Pituitary. There are a number of reports of separation of 2 or more gonadotropic factors from the pituitary, but none of these have been extensively confirmed.

There are a number of points which do not seem to be adequately explained by the two-hormone theory: *a),* LH must be mixed with FSH in order to produce augmentation. If these two are separate hormones augmentation should be obtained when they are injected concurrently but separately. *b),* Augmentation of FSH extracts is produced by proteins and zinc sulfate as well as by LH, from which it would seem that augmentation is a non-specific reaction. *c),* Very pure preparations of mare serum and pregnancy urine give responses identical to those of the original product, showing that there is no separation of two hormones in these cases. *d),* Mare serum produces corpora in hypophysectomized rats (Smith, 29) but not in very young immature female rats. Addition of pituitary LH did not produce corpora, thus indicating that factors other than specific pituitary hormones are involved in determining the response. *e),* Although several methods have been reported for the separation of two or more factors from pituitary, other workers have not been able to repeat these methods successfully. *f),* The type of response obtained with a purified FSH extract is dependent upon the number of injections. *g),* The qualitative as well as the quantitative ovarian response to mare serum is dependent upon the size of the dose.

Some of the points submitted in substantiation of the two-hormone theory are: *a),* Wallen-Lawrence (18) used successive injections of FSH and LH. We have discussed what we consider a more logical explanation of her results. *b),* Fevold, et al (30) claim a specific augmentation of FSH by LH as compared with copper acetate. In these experiments a dose of a combination of FSH and copper acetate was chosen which produced the same ovarian weight as a given dose of FSH plus LH. Then multiples of these doses were used, and the rate of ovarian weight increases compared. It was found that the rate of increase was much greater in the case of FSH plus LH. There are two possible sources of error in this experiment. First, different amounts of FSH were used as starting points in the two experiments, and second, whereas in one case both FSH and LH were increased in the other only the FSH was increased, not the copper acetate. *c),* According to Greep (10) FSH is responsible for the development and maintenance of the germinal epithelium of the seminiferous tubules in the hypophysectomized male rat, whereas LH is necessary for interstitial cell development. However, according to Pfeiffer (31), the male hy-

pophysis secretes only FSH. Likewise, Cutuly, et al (32) have shown that male sex hormone is of some value in maintaining the testis tubules, indicating that it can, to some extent, replace FSH. Evans, et al (3) believe that still another gonadotropic factor, *ICSH,* is responsible for the development of interstitial tissue. *d*), It has been claimed that LH has specific properties in inducing luteinization (3, 4). However, the experiments reported in this paper do not add much support to this contention. LH failed to luteinize follicles developed by mare serum in the 18-day-old rat. Contrary to expectations, FSH alone produced some corpora in the rat of this age. In the 25-day-old rat the addition of $ZnSO_4$, egg albumin or casein to synergist was just as effective in increasing the numbers of corpora as was LH.

However, in the 21-day-old rat one FSH fraction gave a pure follicle-stimulating response when given twice daily for 4 days, whereas LH given in the same manner produced a few corpora. It should be mentioned, however, that corpora were produced by this same FSH alone when the injections were given once daily for 3 days. Thus, it is clear that the manner of administration plays a rôle in determining the qualitative response.

Even though our pituitary preparations were made by Fevold's method, one cannot be assured that our preparations are comparable to his. He has kindly supplied us with some of his preparations. It has been deemed advisable to make further tests on a new preparation before reporting the results on these fractions.

We now feel that there is ample reason for abandoning the view that the gonadotropic action of a given tissue or body fluid is dependent upon the relative concentration of 2 gonadotropic hormones, FSH and LH. Therefore we propose the following as a working hypothesis. The differences in activity of gonadotropic extracts from the several sources are dependent, not upon the relative concentration of FSH and LH, but upon slight variations in the chemical nature or physical state of the hormone. This might involve slight changes in the basic molecule, as is the case with the estrogens or conjugation of the basis molecule with another compound as with thyroglobulin. Furthermore, the possibility of adsorption of the hormone upon some substance must be considered after chemical treatment, for it is known that the addition of certain substances such as protein or zinc sulfate will bring about a modification of the response to some pituitary gonadotropic extracts. The differences between PU, menopause urine, and pregnant mare serum could be explained as being due to a slight variation in chemical nature. The differences in gonadotropic activity of the so-called FSH and LH fractions of the pituitary may be due to physical and/or chemical variations brought about by chemical treatment. However, it is necessary to take cognizance of another fact, namely, that LH fractions inhibit estrous smears when given in conjunction with FSH in the 21-day-old but not the 18-day-old rat. The LH fraction is a crude preparation, and may contain a number of substances not present in the FSH fraction, any one of which may be responsible for this action. Nathanson, Fevold and Jennison (17) have recently attributed this response to LH, but Lahr and Riddle (33) believe the lactogenic factor is involved.

Some may feel that we have not given sufficient attention to the important

mass of data relating to the effect of gonadotropic hormones upon the hypophysectomized animal. Much of the significance of data on the hypophysectomized male used in support of the dualistic theory has been brought into question by the work of Cutuly and associates (32). Judging from the paper by Fevold, et al (30), the response of the hypophysectomized female as regards augmentation is very similar to that of the intact immature female rat. In the hypophysectomized rabbit Foster, et al (7) found that FSH would produce follicular growth and ovulation, but LH had unique properties in maintaining functional corpora lutea. Recently, Greep (34) has shown that LH alone in the hypophysectomized mature female rat accelerates the rate of disintegration of existing corpora, and Evans, et al (35) give evidence in support of the view that lactogenic hormone is responsible for the maintenance of functional corpora lutea. Thus it does not appear to us that the hypophysectomized animal gives clear evidence in support of the dualistic theory.

It does seem certain from the work of Evans, et al (20), and from evidence cited above, that extracts can be obtained which inhibit ovarian activity. Whether any such factor is normally secreted by the pituitary is less certain.

SUMMARY

A series of experiments was devised to determine any possible specific effect of pituitary LH when combined with other gonadotropic fractions. It was shown that when egg albumin was combined with pituitary synergist the augmentation produced was just as great as when pituitary LH was combined with the latter, despite the slight gonadotropic activity of the LH fraction. Zinc sulfate was a much more effective augmenting agent than either egg albumin or LH. All of these augmenting agents increased the number of corpora in the ovaries of the test rats. With a constant dose of synergist, but varying the dose of augmenting agent, it was found that casein produced augmentation in just as low a dose as LH. Furthermore, the M.E.D. of zinc sulfate was much less than that of either of these other two substances. No augmentation was produced when synergist and augmenting agent were injected at different sites in the test animal. The injection of LH, zinc sulfate or egg albumin following an injection of synergist did not augment the effect of the latter. In our experience the use of augmentation as a specific test for LH does not seem justified.

With pituitary FSH extracts the type of response was found to depend on the manner of injection. Although certain extracts gave only follicle stimulation when administered in 8 injections, the same dose produced corpora when given in 3 injections. Some differences between the effects of FSH and LH fractions on 21-day-old rats were observed. Chief among these was the effect on the character of the vaginal smear; LH having the property of inhibiting estrus. LH did not, however, inhibit the production of estrous smears in 18-day-old rats. It was determined that LH could not inhibit the estrus-inducing capacity of injected estrin.

Comparison of the relative effectiveness of various extracts in causing ovulation in mature rabbits and in eliciting an ovarian response in immature rats

led to the belief that the physical state (solubility) is an important factor in determining the character of the response. It was found impossible to augment the effect of any mare serum fraction by any of the augmenting agents previously used, despite the fact that one very potent extract was effective by itself in a dose of 0.005 mg. It had been shown that mare gonadotropic hormone produces only ripe follicles when injected into 18-day-old rats. Combination of the serum with pituitary LH or injection of serum followed by LH did not cause luteinization of these follicles. In a few cases, certain pituitary FSH extracts did produce corpora in the ovaries of these very young rats. Here again, however, the response was mainly follicle-stimulation.

It is concluded from these experiments that, whereas it is possible to obtain from pituitary two gonadotropic extracts, having somewhat different effects, it is not certain that these are due to two separate hormones. Whether the ovarian response shall be follicle-stimulation or luteinization seems to depend to at least as great an extent on the manner of injection and state of the ovary as on the type of extract used. A new hypothesis is suggested as an alternative for the two-hormone theory.

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DIABETIC TRAITS IN A STRAIN OF RATS[1]

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A a result of an investigation of the effects of drugs on the carbohydrate metabolism of rats, control glucose tolerance tests revealed that pedigreed, sexually mature, male rats of the Yale strain[2] exhibited a significantly lower glucose tolerance than rats of the Wistar strain of corresponding age and sex, grown under the same conditions and maintained on the same diet. In view of the extensive use of rats in carbohydrate studies, considerable importance is attached to the questions, *a)*, Which strain has a 'normal' carbohydrate tolerance? *b)*, What criteria justify the designation 'normal' carbohydrate tolerance in the rat?

Relative to these questions, the data here reported cover a period of 22 months and consist of 425 glucose tolerance tests on 240 rats, normal with respect to weight gains and general physical appearance. The age of the animals ranged from 22 days to 14 months. No animal had received drugs at any time except the 22 Wistar rats shown in table 2. These animals received small doses of physostigmine or strychnine 10 to 14 days prior to the tolerance tests, but the tests were not affected by the drugs. Glucose was administered by stomach tube or by intraperitoneal injection.

EXPERIMENTAL METHODS

Only male rats were used, and they were maintained on a uniform diet consisting of a commercial food[3] considered complete but supplemented in our laboratory by fresh vegetables. The food was removed 15 to 16 hours before the test glucose was administered. Water in open dishes was always available.

Blood samples sufficient for measuring 0.1 or 0.2 cc. were drawn from the tail after amputating with sharp scissors a thin segment. The blood was milked from the tail into an oxalated glass capsule of approximately 0.4 cc. capacity. Bleeding was facilitated greatly by a humid atmosphere with a temperature of 26°C. or above. A second, though less valuable, aid was an upright position of the rat attained by using a snugly fitting cylindrical wire mesh cage set at an angle of approximately 60 degrees. Hyperemia was not induced in the tail by irritants. At no time (either during the administration of the glucose or the bleeding) were the animals anesthetized. Blood was

[1] A preliminary report of this work was made before the Physiological Society of Philadelphia, November 15, 1937 (1).
[2] Our original rats of this strain were obtained from the Yale Laboratories of Physiological Chemistry in October 1935.
[3] The company's analysis listed the following: digestible carbohydrate, 48%; digestible protein, 19%; fats, 4.7%; ash, 7%.

drawn before the glucose injection and ½, 1, 2, 3, and 5 hours after. Each bleeding averaged 60 seconds.

When the glucose was administered by stomach tube 3.50 gm./kg. in a 30 per cent solution, warmed to 33°C., was injected through a 7-inch section of a No. 6 uretal catheter. Diarrhea was never observed. When the glucose was administered by intraperitoneal injection, 3.50 gm./kg. in a 10 per cent solution, warmed to 33°C., was injected through a lateral wall of the abdomen using a No. 20 gauge needle.

Blood sugar was determined by the Shaffer-Hartmann-Somogyi method

TABLE 1. GLUCOSE TOLERANCE BY STOMACH TUBE[1]

No. of tests	No. of rats	Age Range	Fasting[2]	Hours after glucose administration				
				½	1	2	3	5
			'True' sugar in terms of glucose per 100 cc. of blood					
		days	mg.	mg.	mg.	mg.	mg.	mg.
Wistar Strain								
15	15	134–200	66±1.2	88±0.8	95±1.0	106±1.4	112±1.3	84±1.6
Yale Strain								
10	10	111–210	82±2.4	125±2.3	130±2.9	131±3.4	123±3.3	92±2.5
14	14	110–241	85±2.0	141±3.2	163±3.8	157±5.2	175±5.8	129±4.4

[1] 3.50 gm. of glucose per kg. in a 30 per cent solution.
[2] Food withdrawn 15-16 hours before control blood.

TABLE 2. DUPLICATION OF RESULTS ON 22 RATS USED IN 4 DIFFERENT EXPERIMENTS SPACED 10–14 DAYS APART (WISTAR STRAIN). INTRAPERITONEAL ADMINISTRATION[1]

No. of tests	No. of rats[2]	Fasting[3]	Hours after glucose injection	
			½	1
		'True' sugar in terms of glucose per 100 cc. of blood		
		mg.	mg.	mg.
22	22	66±1.0	170±3.2	136±2.7
22	22	64±0.67	171±3.2	137±2.8
21	21	66±1.0	172±3.3	134±2.2
22	22	66±0.80	171±3.2	135±2.9

[1] 3.50 gm. of glucose per kg. in a 10% solution.
[2] Age in days—93 to 219.
[3] Food withdrawn 15-16 hours before control blood.

(2)[4] on filtrates prepared by the zinc hydroxide precipitation procedure of Somogyi (3). Values obtained by this method are considered generally to represent 'true' blood sugar. With rats over 60 days of age, 0.2 cc. of whole blood was employed, with younger rats 0.1 cc. was used.

Hemoglobin was determined by the Newcomer method.

RESULTS

Glucose administered by stomach tube (table 1). With the Wistar strain the results are remarkably uniform, the probable error ranging from 0.8 to 1.6.

[4] Reagent No. 50 containing 1 gm. of potassium iodide per liter.

In analyzing the results on the Yale strain, however, one is confronted with individual differences sufficiently large to divide the rats into 2 groups. One group resembles reasonably well the Wistar rats and has been termed 'normal,' the other group has a definitely lower tolerance. A logical division places in the 'low tolerance' group those animals whose blood sugar rose above 140 mg. per cent and on the 5th hour remained above 100, and according to this classification 10 of the 24 rats fall into the 'normal tolerance' division and the remaining 14 into the 'low tolerance' division. In the 'low tolerance' division the average blood-sugar curve exceeds that of the 'normal tolerance' group by the following values: ½ hr., 16 mg. per cent; 1 hr., 33 mg. per cent; 2 hr., 26 mg. per cent; 3 hr., 53 mg. per cent; and 5 hr., 37 mg. per cent. These values, expressed as a percentage of the total rise observed in the 'normal tolerance' group, amout to 30 per cent on the half hour; 62 per cent on the first hour; 47 per cent on the second hour; 119 per cent on the third hour; and 340 per cent on the fifth hour. The average blood-sugar curve of the 'low tolerance' division is more irregular than that of the 'normal' group, and the probable error of the 'low tolerance' division exceeds that of the other group by 50 per cent. On the basis of these analyses the 'low tolerance' division has been termed abnormal or diabetic.

Comparing the 'normal' group of the Yale strain with the Wistar strain, the principal differences are, *a),* a lower fasting blood sugar in the Wistar strain; *b),* a greater tolerance in the Wistar strain; and *c),* a lower probable error in the results from the Wistar strain (table 1). The average maximal rise with the Yale 'normals' was 49 mg. per cent, with the Wistar strain 46. With the 'normal' rats of the Yale strain the blood sugar reached the peak value on the first hour but the plateau, formed by the ½, the 1st, the 2nd and the 3rd hour values, changed little. Between the 3rd and the 5th hour the blood sugar in this group fell 31 mg. per cent, a figure which compares favorably with that of the Wistar strain. In the Wistar strain the blood sugar rose gradually to a peak attained on the 3rd hour and between the 3rd and the 5th hour the fall was 28 mg. per cent.

Glucose administered by intraperitoneal injection. Since the factors modifying the intestinal absorption of glucose are more numerous than those affecting the intraperitoneal absorption, we have employed the intraperitoneal route for most of our experiments. The results obtained by this method confirm those obtained by the intragastric administration on all of the important points. Thus, *a),* The tolerance curves on the Wistar rats may be accurately duplicated; *b),* The probable error in tests on the Yale strain is much larger than that on the Wistar strain; *c),* Sexually mature rats of the Yale strain give tests falling logically into a normal and a diabetic group, the latter group including more than half of the animals.

Darrow and co-workers (4) have studied the intraperitoneal administration of numerous solutions including glucose, and on those points tested (solution volume changes and rate of glucose absorption) our results are confirmatory. With the 10 per cent glucose employed in our experiments the maximal increase in volume attained on the second hour was 100 per cent,

with the isotonic glucose employed in Darrow's experiments (4) the maximal increase in volume was 50 per cent. Injecting the 10 per cent glucose, the volume of the intraperitoneal solution was constant from the 2nd through the 5th hour. Coincident with these volume changes a concentration of the blood, reaching a maximal value of 8 per cent on the 2nd hour and remaining constant through the 5th hour, was observed. On the 5th hour subsequent to the administration of the glucose, 85 per cent had been absorbed. The 2 strains of rats showed similar volume changes and absorption rates. The blood sugar values were not corrected for the 8 per cent blood concentration.

Interval Between Tolerance Tests

During the period of the fast and the test, rats weighing between 300 and 400 gm. lose approximately 15 gm. of body weight, which is not regained until

TABLE 3. EFFECT OF STRAIN AND AGE ON THE GLUCOSE TOLERANCE. INTRAPERITONEAL ADMINISTRATION[1]

No. of tests	No. of rats	Age Range	Fasting[2]	[1] Hours after glucose injection				
				½	1	2	3	5
			'True' sugar in terms of glucose per 100 cc. of blood					
		days	mg.	mg.	mg.	mg.	mg.	mg.
				Wistar				
16	16	48– 53	59±1.6	167±4.8	118±2.5	105±1.2	100±2.1	94±1.7
22	22	93–207	66±1.0	170±3.2	136±2.7	113±1.4	112±0.7	110±0.8
27	7	290–436	67±0.8	165±1.9	131±2.4	109±1.6	115±1.9	110±2.2
				Yale				
20	20	21– 30	63±2.5	180±4.9	119±3.9	102±3.0	99±3.2	86±1.8
17	17	31– 40	55±0.7	179±4.2	115±1.9	105±2.4	103±2.0	81±2.2
16	16	41– 50	61±2.4	181±3.7	131±5.6	111±4.5	103±4.1	97±4.2
21	21	51– 60	64±1.7	215±5.7	166±6.8	131±6.9	127±8.2	122±7.3
21	21	61– 70	72±1.3	202±4.8	167±6.6	145±5.4	147±6.0	127±5.9
20	20	71– 80	74±1.6	216±5.6	185±6.6	173±8.0	177±5.8	168±7.9
20	20	81– 90	78±1.2	237±6.7	210±6.8	185±7.1	180±7.1	155±6.4
37	26	101–140	74±1.5	168±4.3	164±4.9	161±3.8	166±4.8	173±5.2
30	26	141–180	81±2.0	217±5.7	207±6.3	202±8.1	203±8.6	214±9.5
30	25	181–220	78±1.8	194±4.7	189±6.3	172±7.7	186±7.7	177±5.7
22	14	221–303	77±1.3	196±4.8	162±5.0	167±5.6	183±6.8	193±7.8

[1] 3.50 gm. of glucose per kg. in a 10% solution.
[2] Food withdrawn 15-16 hours before control blood.

5 days later. Consequently, we adopted a minimum rest period of 10 days between tests and obtained a remarkable duplication of results with the Wistar strain (table 2). In this table we have recorded only the 30 and 60-minute periods; since the subsequent periods involved changes produced by drugs, however, an inspection of table 3 shows that the first 2 periods involve the greatest probable error and may be considered as the most severe test in comparisons. The results obtained on rats of the Yale strain are more variable than those observed with animals of the Wistar strain (table 3), but the difference is not due to a slower recovery.

Age Factors (table 3)

Wistar strain. In rats under 54 days of age we have found a slightly higher glucose tolerance than in older rats. Factors contributing to this effect may be, *a)*, the higher ratio of liver weight:body weight (5); *b)*, a higher rate of metabolism; *c)*, a modified endocrine balance. At 90 days of age, the rats are sexually mature and their glucose tolerance assumes a value which does not change up to 436 days, beyond which our data do not extend. In senile rats, over 900 days of age, Addison (6) reported pituitary changes; consequently, in this period tolerance changes may appear.

Yale strain. The tolerance curves given by all rats of the Yale strain, age groups 21 to 30, 31 to 40, and 41 to 50 days, approximate closely each other and also the curve for Wistar rats of comparable age (fig. 1). However, the curves in figure 1 suggest that the age group 41 to 50 days has a lower toler-

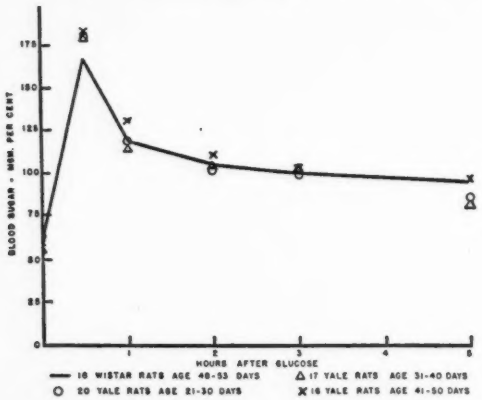
BLOOD SUGAR - MGM. PER CENT
HOURS AFTER GLUCOSE
16 WISTAR RATS AGE 40-53 DAYS
17 YALE RATS AGE 31-40 DAYS
20 YALE RATS AGE 21-30 DAYS
16 YALE RATS AGE 41-50 DAYS

Fig. 1. Glucose tolerance curves of all rats of the Wistar and Yale strains under 53 days of age. Intraperitoneal administration.

ance than the younger groups, and table 3 shows that the probable error of the curves for the 41 to 50 day group is definitely larger than that for the younger animals. In the group 51 to 60 days of age the probable error exceeds that of the 41 to 50 day group, and in this 51 to 60 day group some of the animals give curves sufficiently different from the average to justify the designation 'diabetic.' In all of our analyses the criteria for a normal curve following intraperitoneal injection in either rat strain are, *a)*, the ½ hour blood sugar must be greater than the 1st hour value; *b)*, the ½ hour value must be less than 300 mg. per cent; *c)*, the 5th hour value must be at least 40 mg. per cent below the ½ hour value, or 60 mg. per cent below if the ½ hour value is over 200 mg. per cent; *d)*, the 5th hour blood sugar must be below 180 mg. per cent. These criteria require that the normal curve conform in general to the curves in figure 1. Employing these criteria, figure 3 shows the distribution of normal and diabetic curves in different age groups of the Yale strain. Figure 2 supplements figure 3 and gives the average of the normal curves in

each age group. In the age group 21 to 90 days (fig. 3) each curve represents the reaction of an individual rat, but after age 91 days 2 or 3 curves were sometimes obtained from one animal. The latter results do not distort the data or alter any conclusions. For example, 68 per cent of the curves in the age group 91 to 240 days are diabetic (fig. 3, intraperitoneal injection), but, using a different animal for each curve, 58 per cent of the curves in the same age group are diabetic (table 1, intragastric administration). Considering the smaller number of animals involved in the second group, the results are not significantly different. The data in figure 3 show that the diabetic trait does not appear before 51 days of age, and that during the periods 51 to 60 and 61 to 70 the percentage of diabetic curves in each group is 14. However, beginning with age groups 71 to 80 days the percentage of diabetic curves jumps essential-

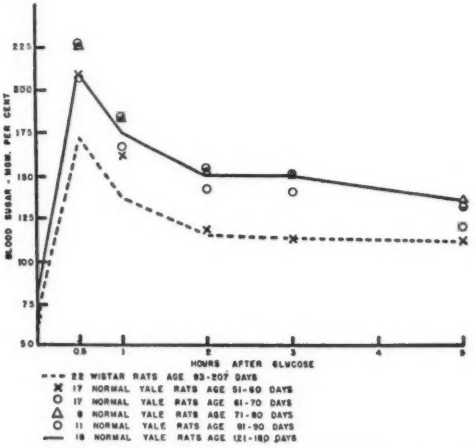

Fig. 2. A comparison of the normal tolerance curves of rats of the Yale strain, age groups ranging from 51-180 days with the average curve of all rats of the Wistar strain, age 93-207 days. Intraperitoneal administration.

ly to the adult level. The age group 71 to 90 days represents the period of sexual maturity.

Tolerance in Adult Rats

The term 'adult' is applied to rats over 90 days of age. A comparison of the tolerances in adult rats of the 2 strains (table 3) shows that the fasting blood sugar of the Yale strain averages 10 mg. per cent more than that of the Wistar strain. In the Yale strain there is no consistent change in the different adult age groups, and in the Wistar strain the only 2 adult age groups studied gave identical curves. An inspection of table 3 impresses one with the fact that the average for no adult Yale group conforms to the criteria for a normal curve. The probable error in this strain demonstrates the inconsistencies in the behavior of these animals, and averages 4 times the probable error of the Wistar strain. In some groups the probable error is 8 times that of the Wistar strain. In addition to the marked differences between the tolerances of indi-

vidual rats of the Yale strain, different tolerances on the same rat may show curves of different types. Our data include tests on 51 animals used 2 or 3 times after they were 90 days of age. Five of these rats always gave normal curves, 24 gave only diabetic curves and 22 gave curves of both types. Usually the normal curves obtained from this last group barely met the requirements for the normal and indicated the diabetic tendency in one or more points.

In figure 2 we have plotted the average of the 18 normal curves of the Yale strain between the ages 121 to 180 days. This group contained 55 curves

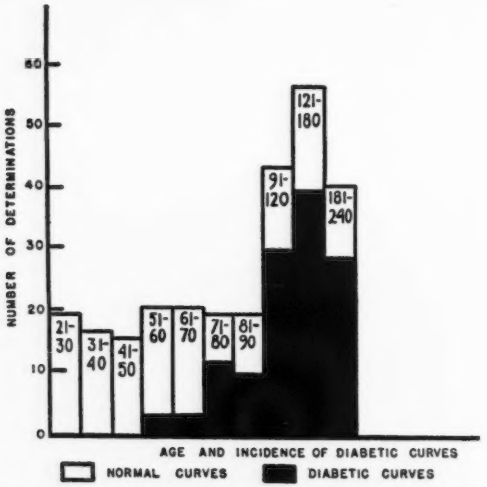

Fig. 3. Distribution of diabetic curves in the age groups of the Yale strain. Figures in the blocks record age in days. Intraperitoneal administration.

on 38 rats. The curve of the normals parallels the curve for adult rats of the Wistar strain, but at each point exceeds the Wistar curve by 25 to 35 mg. per cent of blood sugar. As shown in the same figure the normal values in the Yale strain, age group 51 to 60 days, agree closely with the adult rats of the Wistar strain. The normals in the Yale strain, age group 61 to 70 days, fall between the adult Yale and Wistar curves, and the normals in the Yale groups 71 to 80 days and 81 to 90 days are identical with the adult Yale average.

The 37 diabetic curves in the Yale group, age 121 to 180 days (typical of the adult Yale series) show 3 different types: 15 in type 1; 10 in type 2; and 12 in type 3 (fig. 4).

DISCUSSION

The difference in the behavior of the 2 strains is not due to a bartonella infection. Convincing experiments in our laboratory with bartonella-infected and free animals in each strain have demonstrated that there is no relation between this infection and glucose tolerance.

In seeking other factors responsible for the difference in the behavior of the 2 strains with the glucose tolerance test, one is impressed by the fact that rats of the Yale strain grow faster and larger than animals of the Wistar strain. In our laboratory, where the 2 strains were grown under identical conditions, individuals of the Yale strain weighed at weaning (21 days old) 11 gm. more than rats of the Wistar strain. At 60 days of age the Yale rats weighed 50 gm. more than the Wistars, and at 90 days the Yale lead was 104 gm. After 130 days the weight increase in each strain is slow, but at 360 days rats of the

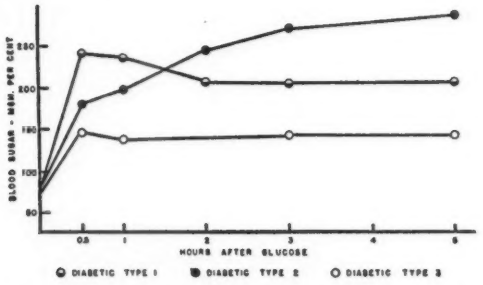

Fig. 4. Types of diabetic curves found in rats of the Yale strain. Intraperitoneal administration.

Yale strain are still larger than animals of the Wistar strain. The recognized relation of the pituitary to growth and to blood sugar places this gland under suspicion, but the literature contains no comparative pituitary studies on the 2 strains.

The data presented demonstrates that the Woman's Medical College colony of rats of the Yale strain is unsatisfactory for studies involving glucose tolerance. If, as is generally believed, the glucose tolerance test is a reliable indicator of a diabetic tendency, the colony contains a high incidence of this trait. From the data available, the incidence of diabetic curves appears to be greater in the Woman's Medical College colony of the Yale strain than in the original stock, and probably reflects the close inbreeding practiced in starting the new colony. Cammidge (7, 8) observed that hyperglycemic and hypoglycemic traits could be accentuated in mice by appropriate breeding.

SUMMARY

Seventy per cent of the glucose tolerance curves of sexually mature, male rats of the Woman's Medical College colony, Yale strain, are diabetic in type.

Animals of the Wistar strain give only normal curves. Diabetic glucose tolerance curves do not appear in rats of the Yale strain under 50 days of age, and the average tolerance curve for these animals is identical with that of the Wistar strain of the same age group. In the Yale age groups 51 to 60 days and 61 to 70 days, 14 per cent of the animals in each group give diabetic curves; in the group 71 to 90 days of age 50 per cent of the animals give diabetic curves; and after 90 days of age 70 per cent of the tolerance curves are diabetic. The probable error for the tolerance curves of the Yale strain

averages 4 times that of the Wistar strain. The average normal tolerance curve of adult Yale rats parallels reasonably well the curve of adult Wistar rats, but at each point exceeds the Wistar average by approximately 30 mg. per cent. The fasting (15-16 hr.) 'true' blood sugar of sexually mature rats of the Yale strain exceeds that of the Wistar strain by 10 mg. per cent. The diabetic trait appears to be inherited.

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ENDOCRINE FACTORS INFLUENCING TUMOR DEVELOPMENT[1]

THE EFFECT OF PROLAN UPON THE MARSH-BUFFALO ADENOCARCINOMA

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The influence of estrin upon the development of spontaneous mammary gland carcinoma in susceptible mice is now well established. The explanation of the mechanism is still controversial, there being three theories: *a)*, that estrin is absorbed by the mammary gland where it exerts a carcinogenic effect similar to that exerted by the carcinogenic hydrocarbons; *b)*, that estrin merely causes hypertrophy of the mammary gland and in so doing increases the number of cancer-susceptible cells; *c)*, that estrin has no immediate effect upon the mammary gland, its action being solely through stimulation of the pituitary to produce hormonal substances which incite mammary gland changes.

In the current estrin experimentation (1, 2), the estrin has always been of exogenous origin, and it has appeared pertinent to the writers, for the following reasons, that the influence of estrin of endogenous origin should be studied. *a)*, There is good reason to suppose that the estrins in the body are in a combined form, not as the free, relatively simple sterols obtained by chemical treatment of the body tissues and fluids. Such combined estrogens may have a negative carcinogenic effect; *b)*, The significance of the influence of exogenous estrin will be diminished if the amounts of estrin required to produce carcinogenesis exceed those which it is possible for the ovaries or other tissue to produce at any one time or for any one period; *c)*, The endogenous production of estrin may lead to defense mechanisms not evoked by its parenteral administration. Diaz, et al. (3) have recently shown that large daily doses of estrin act directly upon the pituitary, causing an exhaustion of gonadotropic hormone production. The ovary is not fundamentally damaged by such treatment but becomes atrophic because of inadequate stimulation. Simultaneous dosage of gonadotropic extract produces ovarian development.

In the experiments reported in this paper parenteral administration of prolan has been utilized to stimulate the ovaries of the Marsh-Buffalo strain to excessive estrin and related hormone production, and the effect upon the development of the spontaneous adenocarcinoma has been noted.

EXPERIMENTAL PROCEDURE

Sixty-six virgins of the Marsh-Buffalo strain, 2 to 3 months of age, were pooled and then divided into a control and dosed colony by random sampling.

[1] These studies were made possible by a grant from the International Cancer Research Foundation, Wm. H. Donner, President.

Once a month for 11 months the dosed colony received a total of 60 units of prolan (follutein)[2] in 4 successive days. Each month for the first 4 months 1 of the dosed mice was sacrificed for examination of the ovaries. Ovarian hypertrophy, amounting to 200 to 300 per cent increase in ovarian weight, was noted for each case. Section revealed the presence of ripened follicles, corpora lutea, and blood points. It is important to note that the repeated prolan dosage neither developed immunity to response nor completely luteinized the ovary, follicles being present in various stages of development. At the time of the development of the mammary tumors and in tumor-bearing mice the ovaries had regressed to the size of the control ovaries. Forty days after the first dosage with prolan 1 mouse died. Autopsy revealed a chocolate ovary $1.2 \times 0.8 \times 0.8$ cm.

The first mammary tumor developed at the age of 9 months. Excluding the 4 mice killed for ovarian section and the mouse which developed the chocolate ovary, there remained 30 control and 31 dosed mice. These are the numbers used in computing the incidence of mammary tumors.

RESULTS AND DISCUSSION

By the 21st month of age, 76 per cent of the controls had developed adenocarcinoma of the breast, 13 per cent had developed lymphosarcoma, and

TABLE 1. INFLUENCE OF PROLAN DOSAGE UPON TUMOR DEVELOPMENT IN VIRGIN MICE OF THE MARSH-BUFFALO STRAIN[1]

Age of mice, months	% which developed or had breast tumors (adenocarcinoma)		% which developed or had lymphosarcoma		% dead of other causes	
	Control	Dosed	Control	Dosed	Control	Dosed
9	3	0	0	3	0	0
10	3	0	0	3	0	3
11	3	3	3	3	3	3
12	10	13	3	3	6	3
13	16	19	3	3	6	6
14	25	26	3	3	6	6
15	36	39	6	3	6	9
16	45	48	6	6	6	9
17	45	52	6	9	6	9
18	51	55	9	13	9	9
19	60	61	13	16	9	9
20	73	61	13	16	9	13
21	76±8	65±8	13	16	9	13

[1] The data are for 30 control and 31 prolan-dosed mice.

11 per cent had died of causes not related to tumor formation. Of the dosed mice, 65 per cent had developed adenocarcinoma of the breast; 16 per cent had developed lymphosarcoma; and 13 per cent had died of causes not related to tumor formation. Of the 2 surviving mice 1 died later of lymphosarcoma and the other was killed. In 1 mouse which developed an adenocarcinoma of the breast the 21st month of life, a lymphosarcoma was also found.

It will be noted that at no time from the appearance of the first tumor,

[2] E. R. Squibb & Sons.

which was in the 9th month of life, to the termination of the experiment was there ever a significant difference in tumor incidence between the control or dosed series (the difference never exceeded the standard deviation of the mean).

Although it is possible to evaluate the prolan unit in terms of estrin units by determining the minimal amounts of each required to produce estrus in immature mice, this factor should not be applied in the present experiment because large amounts of prolan bring about not only the maturing of the follicles but also luteinization, which supposedly through progesterone production finally inhibits estrus. We have found, working with the mouse, as Bennett, et al. did working with the rat, that prolan produces acinar development in the mammary gland, but only in the intact animal. These mammary gland changes induced in our experiment by prolan obviously did not influence carcinogenesis.

Both the estrins and progesterone, when of exogenous origin, have been shown to produce mammary gland hypertrophy, but it is apparent that when similar substances are endogenously produced by the ovary through maximum prolan stimulation they do not lead to increased or earlier carcinogenesis. The enhanced effect which is brought about in these mice by pregnancy is not duplicated in virgins by prolan dosage. It would be premature to discuss the problem further until experiments with the other gonadotropic fractions and their related hormones have been concluded. In fact, it was originally intended to withhold publication of the present experiment until the whole series was complete. Since, however, there exists some apprehension regarding the clinical use of estrogens and their stimulators, such as prolan, the results of the present experiment offer some degree of justification for clinical use of prolan without fear of possible resulting carcinogenesis. In this connection, it should be pointed out that, although 1 of the prolan-dosed mice developed and died with a chocolate ovary, this phenomenon has also occurred in one of our control series, in which 1 of 40 mice developed a large chocolate ovary and died.

SUMMARY

In a series of 61 virgin female mice of the Marsh-Buffalo strain, monthly treatment with prolan, given to produce 200 to 300 per cent ovarian hypertrophy and therefore marked hyperovarian function, failed to influence the onset or incidence of the adenocarcinoma of the breast to which this strain is subject, and which is normally enhanced in breeding animals.

It is concluded that the endogenous production of estrin and its related compounds by the ovary through prolan stimulation does not influence carcinogenesis, and that the production of carcinogenesis by massive doses of estrin (exogenous) may be without physiologic significance.

We are indebted to Richard D. Evans for the histologic reports.

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BLOOD POTASSIUM AND SUPRARENAL GLANDS

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Of the several functions attributed to the suprarenal gland, those which have proved most interesting refer to the regulation of water (1 to 4) and mineral metabolism, especially that of sodium (5 to 11) and potassium (12 to 21).

The study of the chemical composition of blood and urine of suprarenalectomized animals (2, 9), or subjects with Addison's disease (8, 13, 14, 22, 18), shows an increase in the urinary excretion of sodium, and consequently a decrease of this ion in the blood plasma. To this sodium decrease has been attributed the symptoms of suprarenal insufficiency. It has been found possible to cause a decrease in sodium concentration of blood plasma by other means than suprarenalectomy without the appearance of symptoms of the disease (23 to 26). Nevertheless, the ingestion of sodium chloride (8, 14) or, better, of sodium chloride and sodium citrate or bicarbonate[1] prevents the appearance of the symptoms of suprarenal insufficiency and maintains the life of the animals. This treatment is more efficient if accompanied by cortico-suprarenal hormone.

It has been proven recently that an increase in sodium ingestion alone is inadequate, but in addition potassium ingestion must also be reduced (5, 27, 6, 14, 15, 18). It is also interesting, in this connection, that in some animals, such as the opossum and marmot, suprarenalectomy produces an increase instead of a decrease of sodium in blood plasma (28).

Following suprarenalectomy blood potassium increases, and some investigators believe that the observed symptoms could be due to an intoxication by this element (19, 20). An increase of blood potassium has been observed in the cat by Zwemer et al (16, 29, 11), Urechia, Benetato and Retezeanu (17), Bauman and Kurland (30), and by Hegnauer and Robison (7); in the dog by Hasting and Compere (12), in the rat, the mouse and guinea pig by Zwemer and Truszkowski (20), and in man by Loeb (8), Marañon et al (13) and by Thorn et al (22) and others. In the dog, the increase could not be observed if the diet contained less than 200 mg. of potassium (27). The increase of potassium disappears following injection of cortical hormone (14, 22, 11).

Zwemer and Truszkowski (19) believe that all the signs and symptoms of Addison's disease or of experimental suprarenalectomy can be reproduced by continuous injection of potassium in concentration sufficient to main-

[1] This fact has been attributed to the action of the sodium ion.

tain blood potassium at the same level as that observed in suprarenalectomized animals.

We have studied the variation in mineral substances of the blood of normal and suprarenalectomized dogs, the effect of intravenous injections of potassium in such animals, and the influence of demedulation and denervation of the suprarenal gland upon the potassium of the plasma.

EXPERIMENTAL METHODS AND RESULTS

Dogs were used in all of our experiments. In this Institute, suprarenalectomy has been done in 2 stages. First the right adrenal was extirpated via the abdominal route. When the animals recovered (1 or 2 weeks later), the left gland was quickly removed through a lumbar incision under ether anes-

TABLE 1. BLOOD MINERALS BEFORE AND AFTER DOUBLE SUPRARENALECTOMY IN DOGS

Experiment	Wt. of dog, kg.	Time in hours after suprarenalectomy	Milligrams per cent									
			Phosphorus		Chloride		Potassium		Sodium		Calcium	
			Before	After	Before	After	Before	After	Before	After	Before	After
119	9.0	48	4.94	7.27	397.6	352.1	16.2	27.7	374.8	336.8	12.8	11.2
122	11.4	48	4.76	5.26	408.3	369.9	18.4	22.1	402.4	340.8	13.1	12.6
125	6.8	48	4.52	6.05	390.5	330.1	24.7	33.1	372.4	317.4	12.6	11.5
128	13.0	36	3.72	5.19	401.6	353.6	21.1	23.8	405.2	350.8	12.1	13.1
130	9.0	36	4.44	5.92	400.5	350.8	21.0	28.6	405.2	350.8	12.0	12.5
133	12.5	36	3.40	4.65	390.5	340.8	20.8	29.6	370.4	342.8	11.4	10.7
134	9.5	36	4.37	5.25	385.6	315.5	20.3	37.4	365.2	326.8	11.2	10.6
Mean values			4.30	5.65	396.3	344.7	20.3	28.9	385.1	338.0	12.2	11.7
Per cent				+31.3		−13.0		+42.3		−12.2		−4.1
Mean values from 9 normal animals			4.18		389.6		18.9		385.3		11.2	

thesia. Before doing the second operation, a sample of blood was taken from the jugular vein and, 36 to 48 hours after the second gland was extirpated, another sample of blood was taken. Blood coagulation was prevented by means of heparin and used for the determination of sodium, potassium and calcium by the Marenzi and Gerschmann methods (31, 32, 33), chloride by the method of Van Slyke and Sendroy (34) as modified by Eisemann (35), and phosphorus by the method of Fiske and Subarrow (36).

When the general condition was good, the same animals were used to obtain tolerance curves, following intravenous injection of potassium. These animals were quieted with small doses of chloralose (50 to 70 mg. per kg.). All experimental animals were maintained on a diet of meat and milk, to which was added 2 or 3 gm. sodium chloride a day. When they refused meals, sodium chloride was given by means of a stomach tube. Some animals were treated with cortical hormone in the manner described in the protocols of this study.

The demedulation was also done in two stages (technic of Houssay

and Lewis). The left gland was demedulated, and when the animals recovered the right gland was extirpated. These dogs had only the left suprarenal cortex. The blood potassium was analyzed from time to time before and after the operations. Dogs with denervated suprarenals were prepared by cutting the major and minor splachnic nerves and the lumbar sympathetic chain.

Effect of suprarenalectomy on blood minerals. The mineral substances mentioned above were determined in 7 suprarenalectomized dogs before and after the operation. The sample called 'before' was obtained when the animal had only one suprarenal gland, and about a week after the operation (table 1).

The values for the mineral elements of plasma after extirpation of one suprarenal gland are normal. There is a great increase in phosphorus and potassium after total suprarenalectomy. The percentage increase of these elements is approximately of the same order of magnitude. Sodium and chloride decrease.

It must be pointed out that the dogs of experiments *133* and *134* were in a very bad condition, presenting contracture and opistotonos accompanied by rigidity of the fore and hind limbs for short periods of time, and after the appearance of 2 or 3 convulsions they died.

Our results are similar to those obtained by Baumann and Kurland (30) for cats and rabbits, by Zwemer and Sullivan (11) for cats, and by Loeb (8) by Marañon, et al (9) in Addison's disease.

Effect of the extract of suprarenal cortex on blood minerals.[2] In 3 cases the effect of cortico-suprarenal extract was studied. In one experiment, *132,* the extract was injected immediately after total suprarenalectomy, and the administration was continued during 48 hours with a dose of 1 cc. representing 40 gm. of fresh gland per kg. during the first 24 hours, and 0.5 cc. per kg. in the next 24 hours.

In the other two cases (experiments *136* and *137,* after total suprarenalectomy we waited for symptoms of insufficiency to appear and, when the animals refused to eat and move and asthenia was intense, injections of the extract were started with doses of 2 cc. per kg. during the first 24 hours and 1 cc. per kg. in the following 24 hours. All the symptoms disappeared, and complete recovery was observed. In these 2 animals the concentration of mineral substances of the plasma was determined before suprarenalectomy, during the insufficiency and after the administration of the extract. The results are given in table 2.

In the first experiment there was no variation of the mineral elements before and after suprarenalectomy, which means that the extract compensates for the alteration. More significant were the other two experiments, in which the appearance of symptoms of insufficiency were allowed to develop before injecting the extract. In these animals the mineral composition of the blood returned to a normal condition 48 hours after giving the extract.

[2] We owe thanks to Dr. Cartland, from the Upjohn Co., Kalamazoo, Mich., and Drs. Lopetegui and Dominguez, from Buenos Aires, for the supply of the suprarenal extract used in this study.

Complete compensation for the variation of some of the mineral elements of the plasma by the cortico-adrenal extract has been demonstrated in cats by Zwemer and Sullivan (11), in dogs by Harrop et al (2) and by Nilson (15), in rats by Ingle, Nilson and Kendall (37), and in man by Marañon et al (14) and by Thorn et al (22).

Potassium tolerance curves for suprarenalectomized and suprarenalectomized treated (cortico-suprarenal extract) dogs.

Houssay and Marenzi (39) established the tolerance curves for intravenously injected potassium, in doses of 10 to 15 mg. per kg., these injections having been made in 90 seconds.

In the normal dog the maximum quantity that can be injected in this

TABLE 2. EFFECT OF CORTICO-ADRENAL EXTRACT ON MINERAL SUBSTANCES OF THE BLOOD OF SUPRARENALECTOMIZED DOGS

Experiment	Wt. kg.	Condition	Total quantity of extract given, cc.	Milligrams per cent				
				Phosphorus	Chloride	Potassium	Sodium	Calcium
132	10.5	Before	—	3.51	400.4	21.5	363.3	11.8
	9.0	48 hours after extract	27.0	4.17	395.5	23.2	356.8	12.2
136	8.9	Before	—	4.49	373.4	18.5	377.2	11.0
		36 hours after adrenalectomy	—	7.40	364.9	24.8	350.8	11.1
	8.0	48 hours after extract	30.0	4.88	376.3	17.4	379.6	10.4
137	11.2	Before	—	4.18	383.0	20.2	350.8	11.3
		36 hours after adrenalectomy	—	5.93	370.1	26.3	331.2	10.6
	10.8	48 hours after extract	40.0	4.49	386.2	20.5	353.6	9.9

way is 20 mg. per kg. of body weight, but the dose which can best be tolerated is 15 mg. per kg. This quantity of potassium produced a maximum increase in the plasma 2 minutes after the beginning of the injection, and then rapidly decreased to the initial level in 10 to 15 minutes. This means that the injected potassium disappears very rapidly from plasma, and that it is fixed in the liver and muscle has already been established. The curve of disappearance of injected potassium is unmodified in nephrectomized animals, which would signify that the change is not due to a rapid urinary excretion but to a retention by the tissues.

Suprarenalectomized animals are more sensitive to potassium than normals, and with rare exceptions the doses of 15 mg. per kg. of body weight is fatal. Of 4 dogs injected with this dose only 1 survived. If their general condition is good, they can easily tolerate doses of 10 mg. per kg.

There is an important difference in the form of the potassium tolerance curve (fig. 1) of suprarenalectomized animals as compared with normal individuals. The initial increase (2 minutes after the beginning of the injection) is greater in the suprarenalectomized[3] than in the normal animal, and this is followed by a rapid decrease during the first 10 minutes that does

[3] It may be that this is due to a diminished quantity of plasma, but we have not measured it.

not reach the initial value in blood, and remains above for the next 20 minutes, with a tendency to increase at the end of this time. These surves show that the fixation of potassium by tissues is retarded and prevents the return of blood potassium to the normal level.

If the suprarenalectomized animals are treated with cortico-suprarenal extract, the tolerance curve has the same slope as that obtained for normal dogs, i.e., returning to the normal value in 10 to 15 minutes (table 3).

Zwemer and Truszkowski (20) observed that suprarenalectomized rats and guinea pigs had greatest sensitivity to intraperitoneal injections of

TABLE 3. TOLERANCE CURVES FOR NORMAL AND SUPRARENALECTOMIZED DOGS AFTER INTRAVENOUS INJECTION OF POTASSIUM (CHLORALOSE ANESTHESIA)

Experiment	Wt. kg.	Mg. injected potassium per kg. of body wt.	Milligrams per cent						
			Before	At minutes					
				2	5	10	15	20	30
Normals									
91	11.0	10	14.3	17.8	13.9	—	13.1	—	11.4
92	8.3	10	14.9	18.6	15.2	—	14.0	—	13.8
94	24.4	10	16.5	21.2	16.9	—	16.0	—	15.5
93	34.3	15	14.3	21.5	16.9	—	15.4	—	—
98	7.5	15	14.9	27.4	18.9	15.4	—	15.6	14.5
Suprarenalectomized									
129	13.0	10	23.5	33.6	27.2	26.7	—	26.1	26.0
131	9.0	10	27.6	43.4	36.0	34.2	—	32.8	33.6
129	13.0	15	26.3	49.4	34.8	32.2	—	31.8	33.6
Suprarenalectomized treated with cortico-suprarenal extract									
134	9.0	10	22.2	34.7	25.3	24.7	—	23.8	22.8
138	8.0	10	18.7	25.3	22.0	19.2	—	17.8	17.3
139	10.8	10	20.8	29.0	23.4	20.5	—	21.0	20.4

potassium and a normal tolerance if the animals were previously injected with cortico-suprarenal extract.

Zwemer and Truszkowski (38) also studied the modifications of plasma potassium in normal subjects and of individuals with Addison's disease, following oral administration of potassium. When a normal subject is given 10 mg. of potassium chloride per pound, the potassium of the blood is not modified, but the same dose given to individuals with Addison's disease causes an increase in blood potassium one hour after ingestion, and then a return to the normal value in 2 hours. Giving a normal subject twice this amount of potassium chloride results in a small increase in potassium which does not reach the value observed in Addison's disease with half the dose, and a return to the normal value in 90 minutes. These results cannot be compared withour own; however, they indicate a modification of potassium metabolism in Addison's disease.

Effect of demedulation and denervation of the suprarenal gland on plasma potassium. In 3 dogs we determined the plasma potassium before and after cutting the splanchnic nerves and the abdominal sympathetic chain on both sides, with the results presented in table 4. It will be seen that 4 days after denervation there is a small increase of plasma potassium that does not per-

sist but returns to the normal value after 10 days and remains at same level 24 days after the operation.

In 3 dogs without suprarenal medula, the plasma potassium was determined, with the results given in table 5. It is important to remember that these dogs had only the suprarenal cortex of one side. They did not show modification of the plasma potassium during the 6 days following the last operation, but after that time there was a tendency for the potassium to increase. This is explained on the basis of a mild degree of cortico-suprarenal insufficiency, and has been proven repeatedly in this Institute. It disappears in 5 to 10 weeks.

If adrenalin were the substance which maintains the constant level of

TABLE 4. PLASMA POTASSIUM OF DOGS BEFORE AND AFTER EXTIRPATION OF SPLANCHNIC NERVES AND ABDOMINAL SYMPATHETIC CHAIN

Days after operation	Milligrams of potassium per cent		
	Dog *88* 14 kg.	Dog *89* 17 kg.	Dog *90* 13.2 kg.
0	18.2	17.5	18.0
	After extirpation of sympathetic and splanchnics		
4	19.1	19.4	21.2
10	18.0	14.3	14.8
17	16.4	16.9	16.1
24	17.7	17.0	16.1

blood potassium, there should be a decrease instead of an increase of this element in demedulated dogs.

DISCUSSION

The cortex of the suprarenal gland is an essential factor in the mobilization and utilization of potassium by the organism. These results and others published elsewhere (39, 40, 41) have led us to conclude that the problem of potassium metabolism after suprarenalectomy can be explained as a disturbance in the equilibrium between the potassium of plasma and that of tissues.

It has been clearly demonstrated that suprarenalectomy produces a decrease of sodium and an increase of potassium of the blood but if we attribute the sodium diminution to a urinary excretion (8) we cannot say that the only factor of potassium increase is a diminished urinary excretion of this element.

Several hypotheses can be formulated to explain the increase of plasma potassium, but it is clearly not due to a greater ingestion of the potassium, because fasted animals, or those maintained on an uniform diet after suprarenalectomy, will develop it in spite of ingesting a smaller quantity of food. On the other hand, potassium administered orally, or injected intra-peritoneally or intravenously is more toxic to suprarenalectomized animals.

In normal dogs we demonstrated (39) that intravenously injected potassium was fixed especially by the liver and muscle, and this explains its rapid disappearance from the circulation with a simultaneous increase of tissue

potassium. Muscle is the tissue that takes up most the potassium, because hepatectomy does not modify the utilization curve of injected potassium.

In unpublished experiments we have been able to demonstrate that in suprarenalectomized rats and toads there is a great decrease of heart and liver potassium, but only a very small decrease or normal values for muscle potassium. Truszkowski and Zwemer (16) demonstrated in cats a decrease in heart potassium, but in muscle there was a small decrease of the same ion if the calculation was made on the basis of fresh tissue, and an increase when calculated for dry tissue. This fact is explained because of the greater water content of tissues of suprarenalectomized animals. From these results we conclude that the cause of the increase in blood potassium can be due to a liberation of this element, particularly from liver and heart, and perhaps from other tissues. In the muscle there is not a very important variation. If this hypothesis for the origin of the increase of blood potassium is admitted, it also obviously cannot be explained on the basis of a kidney which is functioning normally, since in such a case we should expect an increase in the urinary excretion of potassium that would bring the blood concentration to a normal value.

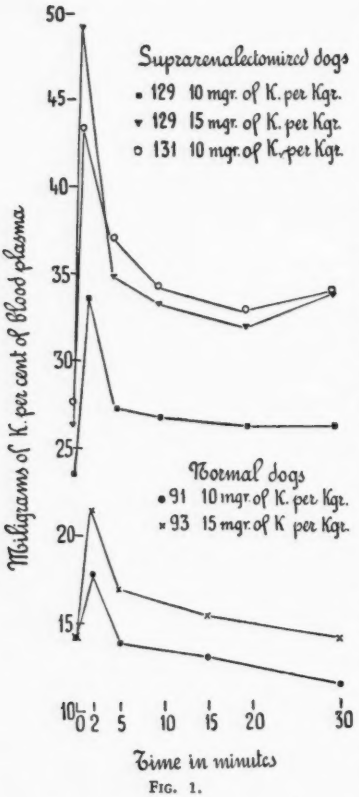

FIG. 1.

For these reasons we must take in account 2 factors for the explanation of potassium increase, *a)*, that the tissues of suprarenalectomized animals do not fix potassium in the same manner as normal controls and, *b)*, that the kidney does not regulate the excretion of this substance as is done normally in maintaining a constant blood level.

Cortico-suprarenal extracts normalize all of these modifications. The active principle of this extract is a factor necessary for, *1)*, binding potassium to tissues, *2)*, regulating the equilibrium of potassium between tissues and plasma, and *3)*, simultaneously regulating the renal excretion of potassium.

The action of the suprarenal gland on potassium is not due to medulary secretion, because the extirpation does not modify the concentration of blood potassium. Nevertheless, there is a small increase of potassium which occurs much later after demedulation than in suprarenal insufficiency.

Our previous results, and those given in this paper show that, in the

TABLE 5. PLASMA POTASSIUM IN DEMEDULATED DOGS

Days after operation	Milligrams of potassium per cent		
	Dog *82* 17 kg.	Dog *85* 18 kg.	Dog *87* 12.3 kg.
0	14.7	18.4	16.9
	Extirpation of the medulla of the left suprarenal gland		
5	—	15.9	17.7
6	14.0	—	—
12	—	15.6	17.5
13	14.6	—	—
	Extirpation of the right suprarenal gland		
5	—	—	17.5
6	18.2	18.8	—
12	—	—	19.9
13	24.4	20.5	—

regulation of plasma potassium, two suprarenal factors are involved, *a)*, the cortico-suprarenal hormone, an agent that regulates the fixation of potassium by tissues, the excretion by the kidney and the equilibrium between plasma and tissue potassium, and, *b)*, adrenalin, which acts as a factor for the rapid mobilization of liver potassium.

CONCLUSIONS

In the dog, suprarenalectomy produces a decrease of sodium and chloride, and an increase of potassium and phosphorus of the plasma. These modifications disappear, and the chemical composition of the blood returns to normal when cortico-suprerenal extract is administered. The intravenous injection of 10 to 15 mg. of potassium per kg. of body weight in normal dogs produces a rapid increase of plasma potassium that returns to normal values in 10 to 15 minutes. Suprarenalectomized dogs are more sensitive to potassium injections than normals. The intravenous injections of 10 mg. of potassium per kg. of body weight, in suprarenalectomized dogs, produces greater increase of blood potassium than in normal controls, and the initial level is not reached after a period of 30 minutes.

The extirpation of the sympathetic abdominal chain and splanchnic nerves does not modify plasma potassium. Demedulation of one suprarenal gland and the extirpation of the other does not produce immediate effects on plasma potassium, and later, between the 6th and 13th days, there is a small increase of this substance attributable to the development of a mild degree of adrenal insufficiency.

The factors regulating plasma potassium concentration are discussed, and the most probable reasons for the increase of this element are given. A hypothesis is presented to explain the mechanism of potassium metabolism on the basis that the cortico-suprarenal hormone is a factor which regulates the fixation of potassium in tissues, its urinary excretion and its equilibrium between plasma and tissues. Adrenalin is a factor for the rapid mobilization of liver potassium.

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THE EFFECT OF TESTOSTERONE-PROPIONATE ON THE X-ZONE OF THE MOUSE ADRENAL

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Masui and Tamura (1) described the zona reticularis of the mouse adrenal as being well-developed and showing peculiar characteristics. Howard-Miller (2) renamed this inner zone of the cortex the 'X-zone' and described the differences between the adrenals of male and female mice. She traced the changes taking place here during the life history of the animals, and proved the transitory character of the X-zone. Deansley (3) confirmed this age-sex dimorphism of the X-zone. The possible analogy of the mouse X-zone to the inner zone of the human adrenal reticularis at birth (4) has stimulated interest in this structure. The fact that the X-zone in the mouse exhibits an age-sex relationship suggests its dependence upon male and female hormones. It is thought to be andromimetic in function (5).

The male and female X-zone both begin development at 9 to 12 days of age and continue to develop until maturity (30 to 32 days). At this time the X-zone of the male rapidly disappears by a process described as a cytolysis of the X-zone cells. Compression of the former X-zone occurs, and a connective-tissue capsule is formed around the medulla. In the nulliparous female, the X-zone continues to increase in size until 45 to 55 days of age, at which time vacuoles begin to appear within the zone. This vacuolation increases with age until the entire X-zone collapses and a condition similar to that found in the mature male is reached. At the onset of pregnancy, the X-zone disappears rapidly within 6 to 8 days. If males are castrated before maturity, the X-zone persists and becomes similar to that of young adult females. Ovariectomy does not appear to affect the female X-zone.

Martin (6) found that injection of testicular hormone, over an extended period, into male or female mice possessing an X-zone produced a uniform effect, in that "a total or marked disappearance resulted." However, he fails to give the details of his experiments. Poll (7) states that a male hormone, which he calls 'proviron,' causes X-zone disappearance in both normal and castrate mice. Burrows (8) suggested that the presence of the X-zone may be influenced by testosterone. Cramer and Horning (9) state that testosterone causes disappearance of the X-zone in the castrate male mouse and young mature females.

Our work substantiates previous findings and adds more information as to the reaction of normal, castrate, and spayed mice of different ages to varied dosages of testosterone propionate given over short periods of time.

MATERIALS AND METHODS

The 247 albino mice (141 experimental and 106 control animals) used in this experiment were of the 'hygenic strain'[1] and were bred and raised in this

TABLE 1. NORMAL ANIMALS

Group	Dosage mg.	No. Inj. (daily)	Total Hormone mg.	Period Effect days	Experimental Males	Experimental Females	Control Males	Control Females
1	0.075	6	0.30	3	1	3	3	2
	0.20	9	1.80	3	5	7	3	5
	0.20	2	0.40	4	4	6	3	3
	0.40	1	0.40	5	3	1	2	1
	0.10	2	0.20	5	8	6	3	2
					21	23	14	13
2	0.075	6	0.30	3	2	1	2	2
	0.20	6	1.20	3	3	0	3	0
	0.20	9	1.80	3	8	9	5	7
	0.40	1	0.40	5	0	8	0	4
					13	18	10	13
3	0.20	6	1.20	3	4	1	4	1
	0.20	12	2.40	6	10	6	5	7
	0.20	12	2.40	3	2	2	1	2
	0.20	7	1.40	2	0	3	0	2
	0.20	5	1.00	2	0	4	0	2
	0.40	1	0.40	5	0	1	0	1
					16	17	10	15

TABLE 2. OVARIECTOMIZED FEMALES

Group	Dosage mg.	No. Inj. (daily)	Total Hormone mg.	Period Effect days	Experimental animals	Non-operated controls	Operated Controls	Total Controls
2	0.40	1	0.40	5	5	2	2	4
3	0.20	2	0.40	3	2	2	2	4
	0.40	1	0.40	5	4	1	1	2
					11	5	5	10

TABLE 3. CASTRATED MALES

Group	Dosage mg.	No. Inj. (daily)	Total Hormone mg.	Period Effect days	Experimental animals	Non-operated controls	Operated controls	Total controls
3	0.20	2	0.40	3	3	1	1	2
	0.20	3	0.60	3	6	2	2	4
	0.20	4	0.80	2	6	3	5	8
					15	6	8	14

laboratory. This strain is said to have been inbred over a period of 8 years. The ages of all animals were definitely known. All experimental animals were

[1] Purchased from the Research Supply Company, Philadelphia, Pa.

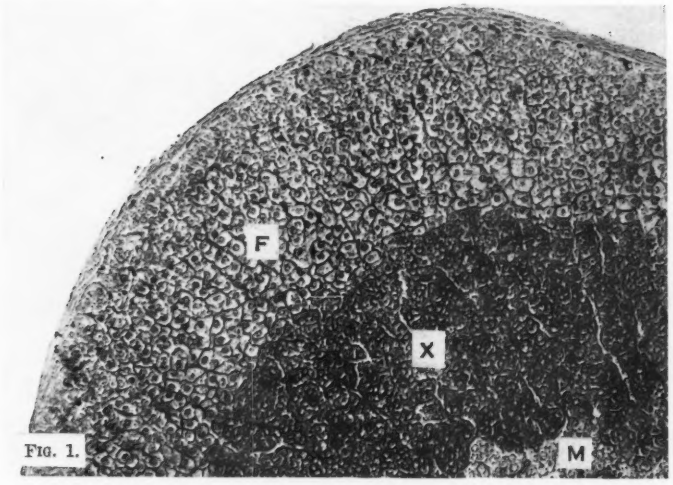

FIG. 1.

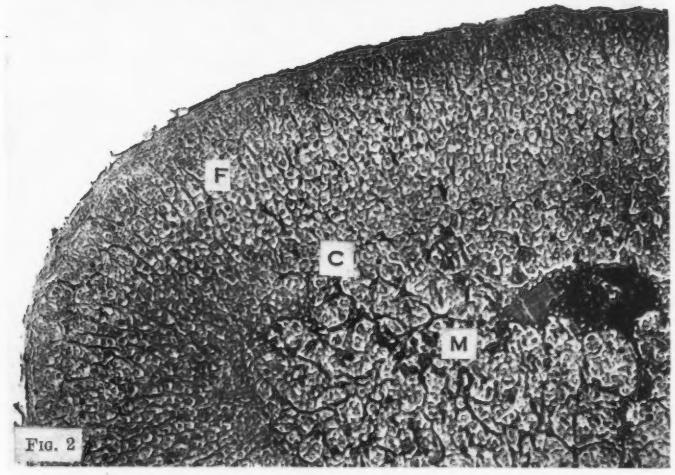

FIG. 2.

Fig. 1. Normal adult adrenal. The X-zone (X) appears as a broad band of darkly staining tissue between the fasciculata (F) and the medulla (M). x115.

Fig. 2. Testosterone treated litter mate of that shown in fig. 1. Note the absence of the X-zone which has been reduced to a connective-tissue band (C) which lies between the fasciculata (F) and the medulla (M). x100.

compared with litter-mate controls. A standard procedure for killing was observed, i.e., anesthesia with ether and bleeding of the animals by severing of the femoral arteries.

The histological procedure for studying the adrenal glands included fixation with Zenkers' formol and Allen's B-3 (urea being omitted), sectioning by the paraffin method, and staining with Delafield's haematoxylin combined with eosin or acid fuchsin. A modification of the Ponceau-G and aniline blue stain described by Broster and Vines (10) was found to give excellent X-

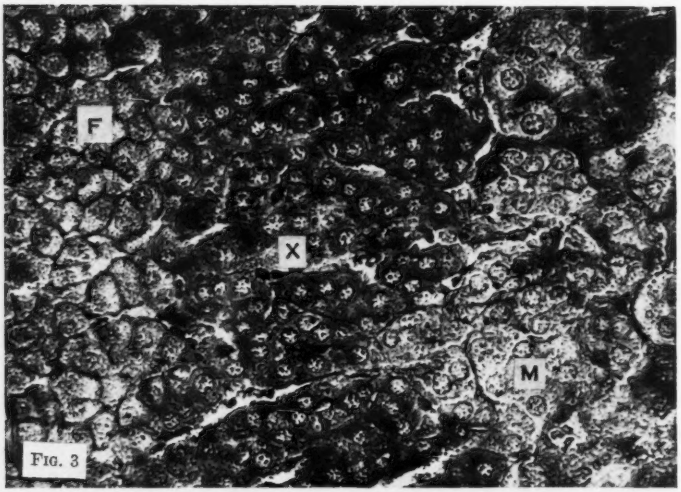

FIG. 3

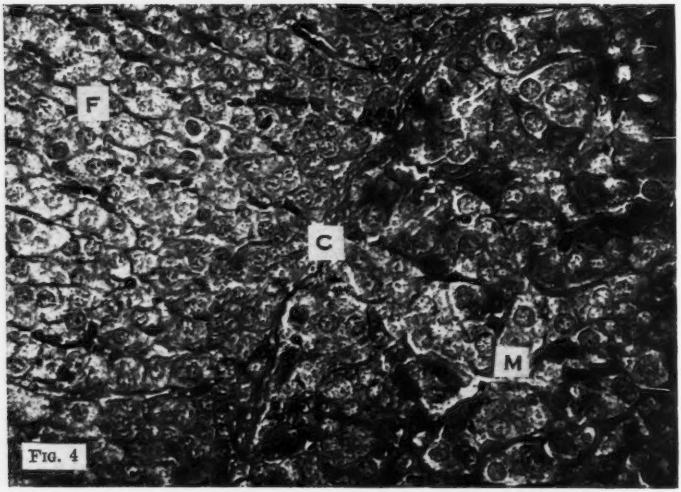

FIG. 4

Fig. 3. High-power view of the normal adrenal shown in fig. 1. The fasciculata (F) appears on the left, the medulla (M) on the right, and the X-zone (X) interposed between these areas. x365.

Fig. 4. High-power view of the experimental adrenal shown in fig. 2. Note the connective tissue band (C) separating the fasciculata (F) and the medulla (M). This band represents the remnant of the X-zone tissue. x365.

zone differentiation, although they failed to obtain a 'fuchsinophil reaction' with normal mouse adrenal tissue. This technic was more satisfactory following bichromate fixation.

The male hormone used was the synthetic testosterone-propionate preparation, oreton.[2] This preparation was dissolved in sesame oil in the concentration of 5 mg. per cc. of oil. The oil was administered by injection into the gluteal muscles, ordinary aseptic precautions being observed. The quantities of hor-

[2] Supplied through the courtesy of Dr. Max Gilbert, of the Schering Corporation.

mone injected and the periods of treatment are indicated in tables 1, 2 and 3. 'Period of effect' is the interval of days between the cessation of treatment and sacrifice of the animals.

Since X-zone activity is associated with age, 3 age levels were established for experimentation. *Group 1.* Mice between 9 and 15 days of age in which the X-zone was beginning to develop. *Group 2.* Adolescent mice 24 to 28 days old in which the X-zone was approaching its prime. *Group 3.* Adult mice over 40 days of age in which the X-zone, in the males, had disappeared or, in the females, was beginning degeneration (vacuolation). Ovariectomy was performed on females of *groups 2* and *3* (table 2). Castration of males in *group 3* (table 3) was performed prior to maturity.

RESULTS

Testosterone treatment of adolescent and adult nulliparous females, normal and ovariectomized, caused a rapid disappearance of the X-zone (tables 1 and 2). Unilateral adrenalectomy was performed on the ovariectomized females and the corresponding litter-mate non-operated controls prior to beginning treatment, to determine the comparative adrenal conditions. No effect of ovariectomy was observed. This agrees with the observations of Deansley (3).

The X-zone disappearance, experimentally induced, appeared to be one of rapid cytolysis of the cells, beginning at the cortical X-zone border, as normally occurs in the male. A single injection of 0.4 mg. of hormone was sufficient to cause X-zone disappearance by the 6th day after treatment. This change is shown in figures 1 to 4. The time period required for this change corresponds with that taken for the disappearance which normally occurs at the onset of the first pregnancy.

Castration of male mice, performed prior to maturity, it known to result in a persistence of the X-zone. Although no X-zone has been observed to persist normally in this strain after 32 days of age, castration as late as 28 days was found to prevent X-zone disappearance. In these experiments, all castrations were performed between 20 and 28 days of age. Unilateral adrenalectomy was performed on the castrate and non-castrate litter-mate controls as well as experimental animals prior to beginning treatment, to determine true persistence of the X-zone due to gonadal removal. Castrate animals were also maintained as operated controls. Testosterone treatment was found to cause X-zone disappearance in normal adolescent males and castrate adult males (tables 1 and 3). The normal adult male adrenals were unaffected.

Normal young (*group 1*) mice, table 1, showed a variation in response. If treatment was begun at 8 to 9 days of age, the X-zone formation was inhibited or prevented. However, if injection was begun at 15 to 16 days of age, when the X-zone was more in evidence, complete degeneration did not take place, with the amounts of hormone injected. An area of degenerative tissue was observed at the medullary border of the X-zone which extended toward the periphery or cortical edge in varying degrees.

Injection of pure sesame oil, without hormone, into 7 females and 7 males, which were compared with the same number of normal litter-mate animals, failed to cause any variation in adrenal condition.

DISCUSSION

Rapid disappearance of the X-zone at the time the testes become functional, and persistence of the X-zone when this normal source of male hormone is removed, points to this hormone as being responsible for normal X-zone disappearance. This is confirmed by the X-zone disappearance in our adult castrate males following testosterone treatment. The prevention of X-zone formation in young animals, disappearance in adolescent animals, and adult females also points to the male hormone as the inhibiting substance. In females, it is possible that male hormone normally secreted in small quantities after maturity (11) is responsible for the gradual disappearance of the X-zone. X-zone disappearance in castrate and ovariectomized animals, following treatment, shows that testosterone propionate acts as a substitute for the normal secretion, and not merely as a gonadal stimulator.

The small amounts of hormone and the short duration of treatment necessary to cause X-zone disappearance indicates the sensitivity of this tissue to male hormone. The time period required for effect corresponds closely to that which has been observed at maturity in the male and initial pregnancy in the female.

Although, as Howard-Miller (2) has suggested, the use of a standardized strain of animals has lessened the variability of adrenal conditions, the differences noted by us between litters emphasizes the necessity of litter-mate controls for comparison with all experimental animals.

SUMMARY

A study has been made of the adrenal glands of 247 albino mice (141 experimental and 106 control animals) of a standard strain maintained under standard conditions. Variations in adrenal conditions were found to be fewer in mice of a standardized strain, but were still found in sufficient numbers to require litter-mate controls for experimental comparison.

Testosterone-propionate treatment over short periods of time has been found to cause X-zone disappearance in normal adolescent males and females, adult females, castrate adult males and adolescent and adult ovariectomized females. X-zone formation has been prevented in young male and female mice. The time required to cause experimental disappearance of the X-zone has been found to correspond to the periods required for normal disappearance.

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THE EFFECTS OF CERTAIN GONADAL AND GONADOTROPIC HORMONES ON THE GESTATION PERIOD OF THE RAT[1]

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As early as 1926, Teel (1) found that gestation was prolonged in rats injected with a crude alkaline extract of beef hypophyses. He stated that the fetuses lived up to or beyond term and then died within the uterus due to a failure of the birth mechanism. Hain (2), D'Amour and Dumont (3), Evans and Simpson (4) have obtained the same results with relatively crude unfractionated hypophyseal extracts. Hain (2) reported prolonged gestation with phyone, but Watts (5) showed that GH free of gonadotropic substances would not inhibit parturition. Hoopes and King (6) prevented parturition with prolan B. It seems, therefore, that this action is associated with the gonadotropic principle, but it is still not known whether parturition is inhibited by the gonadotropic principle directly or whether it acts indirectly through the ovaries. It was hoped that the following experiments would contribute some further data as to the manner in which the birth mechanism is disturbed by gonadotropic substances.

METHODS AND RESULTS

Pregnant albino rats were injected with various extracts during the later part of gestation. Pregnancies were dated by taking vaginal smears of stock rats in the evening and placing all those showing proestrus in with the males. The next morning these females were examined for vaginal plugs and sperm. Beginning with the 21st day all animals were examined several times a day. In this way the length of the gestation period could be determined within about 12 hours. Therefore only animals parturating more than 24 hours beyond the expected time were considered delayed, and 23 days must be considered within the normal range. However, control animals of this colony timed by the above method never parturated more than 12 hours before or after 22 days.

The period of injection is given in the tables, the days recorded being inclusive. When parturition occurred, the day and number of young are given. When gestation was prolonged to the 25th day or beyond, some rats died and others were killed to terminate the experiment. At autopsy the number, size and condition of young in the uterus, the condition and attachment of the placentae and the weight and description of the ovaries were recorded.

Follicle-stimulating and Luteinizing Hormones. Although prolonged ges-

[1] Aided in part by a grant from the Wisconsin Alumni Research Foundation.

tation has been shown to result from injections of gonadotropic hormones, no work has been done with the follicle-stimulating and luteinizing fractions. If gonadotropic extracts act directly, it was considered possible that such activity could be associated with one particular fraction and, if they act indirectly through the ovaries, one might discover how this occurs since the 2 fractions have such different effects on the ovaries of immature animals.

The FSH and LH used in this investigation were prepared by Fevold (7). FSH by itself in relatively large amounts produces only follicles in immature

TABLE 1. RATS TREATED WITH FOLLICLE-STIMULATING HORMONE

Animal	Period of Injection (Days)[1]	Amt. per Day	Termination	Ovary Wt.
59	7	1 gm.	Killed, 27 days	190 mg.
60			Killed, 27 days	293 mg.
8	7	0.4 gm.	Died, 26 days	157 mg.
15			Died, 26 days	165 mg.
7	7	0.2 gm.	Killed, 33 days	136 mg.
11			Killed, 26 days	100 mg.
12			Killed, 25 days	180 mg.
16	16	0.2 gm.	Died, 26 days	139 mg.
17			Died, 24 days	120 mg.
34	16	0.1 gm.	P[2] 5 live, 22 days	
35			P 2 live, 22 days	
26	18	0.2 gm.	Died, 25 days	100 mg.
27			Killed, 27 days	71 mg.
28			P 4 live, 23 days	
56	12–20	0.4 gm.	P 7 dead, 28 days	105 mg.
57			Born and eaten, 28 days	150 mg.
54	12–18	0.4 gm.	Killed, 28 days	100 mg.
55			Killed, 26 days	101 mg.

[1] In all cases where only one day is given, injections were begun on that day and continued to the 25th day or until parturition, if parturition occurred, before the 25th day.
[2] Parturated.

rats, whereas LH has no effect alone, but augments the action of FSH and results in luteinization when injected with it. The doses in tables 1 and 2 are given in equivalents of dried pituitary glands. One-fourth of a gram equivalent of FSH produced 25 mg. ovaries in 21-day-old rats. Injections were made in 0.25 cc. amounts of aqueous solution twice daily.

The results summarized in tables 1 and 2 show that either FSH or LH prolongs gestation and the later in pregnancy injections are begun the larger the daily dose required to inhibit parturition. For example, 0.1 gram of either FSH or LH is effective from the 12th day on, but not from the 16th day on. The ovaries in all cases were of the mulberry type, having many corpora lutea of different sizes and only a very few small follicles. The ovary weights were much higher than in uninjected pregnant animals just before parturition. In 6 uninjected pregnant rats killed on the 21st day, the ovary weights ranged

from 46 to 71 gm., contained a number of nearly mature follicles, and from 8 to 12 large corpora lutea of uniform size.

The fetuses of all animals injected with FSH or LH, except *No. 7, 11* and *60,* were about term size (40 mm.) or larger. These 3 exceptions received large amounts of FSH and injections were started on the 7th day. Apparently the fetuses died and resorption had started shortly after injections were begun. This agrees with the findings of Hain (2), who found injections of pituitary extract during the first half of pregnancy resulted in

TABLE 2. RATS TREATED WITH LUTEINIZING HORMONE

Animal	Period of Injection (Days)[1]	Amt. per Day	Termination	Ovary Wt.
9	7	.05 gm.	Killed, 28 days	147 mg.
13			Killed, 25 days	135 mg.
14			Killed, 25 days	165 mg.
36	12	.1 gm.	Killed, 27 days	152 mg.
37			Killed, 27 days	185 mg.
38	16	.2 gm.	Killed, 27 days	188 mg.
63			Killed, 28 days	170 mg.
64			Killed, 27 days	191 mg.
23	16	.1 gm.	P[2] 7 live, 23 days	
61			P 8 live, 22 days	
62			P 7 live, 23 days	
70	12–20	.2 gm.	Killed, 27 days	173 mg.
71			Killed, 27 days	135 mg.
52	12–20	.1 gm.	P 7 live, 23 days	
53			P 8 live, 23 days	
68	16–20	.3 gm.	Killed, 28 days	110 mg.
69			Killed, 28 days	129 mg.

[1] In all cases where only one day is given, injections were begun on that day and continued to the 25th day or until parturition, if parturition occurred, before the 25th day.
[2] Parturated.

early death of the fetuses. In some cases, where gestation was prolonged, the fetuses were unusually large, indicating continued development beyond normal term. As an example, a fetus of animal *64* weighed 9.5 gm. and had a crown rump length of 55 mm.

The typical condition of animals injected with sufficient FSH or LH to prolong gestation is as follows: the vaginal smear remains as in normal pregnant animals up until about term when blood appears in the vagina and may continue until the animal dies or is killed. With the bleeding, the animal begins to show signs of distress and weakness. At autopsy the ovaries are abnormally large and contain many corpora lutea with few, if any, visible follicles. The uterus contains blood and the placentæ are only poorly attached to the wall of the uterus. The fetuses are of term size or larger and partly resorbed. There is no observable difference between animals injected with LH and animals injected with FSH.

Unfractionated Anterior Pituitary Extract and Hemin. McShan and Meyer (8) found that hemin very markedly increased the gonadotropic effect of unfractionated pituitary extracts. This offered an opportunity to see if prolongation of gestation depended upon the amount of gonadotropic extract injected, or if it depended upon the effect of the extract on the ovary. Injections were made in 0.5 cc. amounts of aqueous solution once daily. Fifty mg. equivalent of the unfractionated APE employed in this experiment gave ovaries weighing 30 mg. when injected into 21-day-old rats. The results in table 3 show that 12 mg. per day of the extract from the 16th day to

TABLE 3. RATS TREATED WITH UNFRACTIONATED PITUITARY EXTRACT AND WITH HEMIN

Animal	Period of Injection (Days)	Amt. per Day	Termination	Ovary Wt.
116	16–24	25 mg. APE	Died, 26 days	152 mg.
117	16–24		Died, 26 days	163 mg.
141	16–22		Killed, 25 days	97 mg.
4 rats	16–22	12 mg. APE	Parturated at term	
5 rats	16–22	3 mg. hemin	Parturated at term	
121	16–22	12 mg. APE and 2 mg. hemin	Died, 23 days (while parturating)	83 mg.
124	16–24		Killed, 25 days	127 mg.
125			Killed, 26 days	307 mg.
126			Killed, 26 days	220 mg.
127			Killed, 26 days	290 mg.

term will not inhibit parturition but can be made to do so by adding 2 mg. per day of hemin which in itself will not prolong gestation.

Animals receiving 25 mg. of extract per day and those receiving 12½ mg. of extract plus 2 mg. of hemin per day were the same in all respects as those receiving sufficiently large amounts of FSH or LH. Gestation was prolonged in the same manner, the fetuses lived up to or beyond term, and the ovaries were of the mulberry type.

Pregnant Mare Serum. FSH, LH, unfractionated APE, and subminimal doses of unfractionated APE plus hemin inhibited parturition and all produced heavily luteinized ovaries with few, if any, macroscopically visible follicles. Pregnant mare serum is known to produce ovaries with large follicles as well as corpora lutea, and has not been shown to contain any hormones other than the gonadotropic. Therefore, to determine if the mulberry type of ovaries were essential to inhibit parturition, and in order to eliminate the possible effect of non-gonadotropic hormones, which are known to be present in most pituitary extracts, 0.1 cc. to 0.4 cc. amounts of pregnant mare serum were injected into rats daily from the 16th day of gestation to term. Two-tenths of a cc. of the pregnant mare serum used gave ovaries weighing 30 mg. when injected into 21-day-old rats. The serum was diluted with water so that all doses were in 0.5 cc. amounts injected once daily.

The ovaries of pregnant animals injected with pregnant mare serum contained more corpora lutea than found in uninjected pregnant animals, but they also contained many large follicles and, in animal *139* of table 4,

a large part of the excess weight was due to a number of extremely large cystic follicles. One R.U. of gonadotropic substance is considered to be that amount which produces a 100 per cent increase in ovarian weight when injected into immature rats. When measured in rat units a greater amount of pregnant mare serum than of unfractionated APE is required to inhibit parturition. As seen in table 4, 0.2 cc. of serum, or 1 R.U., will not prolong gestation, whereas 25 mg. or ½ R.U. of unfractionated APE will.

Extracts of Corpora Lutea. From the foregoing experiments, it would seem that a heavily luteinzed ovary is correlated with prolongation of gestation when gonadotropic substances are injected into pregnant rats. This sug-

TABLE 4. RATS INJECTED WITH PREGNANT MARE SERUM

Animal	Period of Injection (Days)	Amt. per Day	Termination	Ovary Wt.
122	16–20	.1 cc.	P[1] 4 dead, 2 live, 20 days	83 mg.
123	16–22	.1 cc.	Killed, 24 days (young had been born and eaten)	71 mg.
128	16–22	.2 cc.	P 7 live, 1 dead, 22 days	86 days
129	16–22	.2 cc.	P 7 live, 1 dead, 23 days	139 mg.
139	16–23	.4 cc.	Killed, 25 days	588 mg.
145	16–22	.4 cc.	Killed, 25 days	160 mg.

[1] Parturated.

gests that a hormone produced by corpora lutea in some way inhibits parturition. Nelson et al (9) reported prolonged gestation by injecting an extract of corpora lutea, but as this had not been confirmed with the more purified extracts now used, an experiment was set up to see if injections of progestin could inhibit parturition in the same manner as did gonadotropic substances. The extracts used were highly purified and very active in producing progestational changes in the rabbit uterus. Injections were made in 0.25 cc. of oil once each day from the 16th day to term. The amounts used varied from 0.5 to 3 Rb.U. (Corner-Allen) per day and 2 different extracts were used, one prepared by H. L. Fevold, and one by R. K. Meyer.

The results show that progestin does not inhibit parturition when given during the later part of pregnancy even in doses as high as 3 Rb.U. per day. Three Rb.U. represents about 150 gm. of lutein tissue, or three-fourths the weight of an adult rat. It may be that more progestin than that is produced daily by a rat ovary, but it does not seem likely. Since the earlier extracts of corpora lutea contained estrin, theelin was added to progestin in an attempt to reproduce the older extracts. The mixtures of 6 and 10 R.U. of theelin to 1 Rb.U. of progestin were indicated by the determinations of estrin in progestin extracts made by Allen and Meyer (10). These mixtures of progestin and theelin had no more effect than progestin alone.

In the paper by Nelson et al (9), procedures of extraction were published for 2 extracts with which they had obtained prolonged gestation. One of the extracts was a crude methyl alcohol extract injected in aqueous suspension; the other was a methyl alcohol extract purified with benzene and

acetone and injected in oil. Both these extracts were prepared in exactly the manner described, and tests were made to see if they would inhibit parturition in the rat. The amounts in gram equivalents were greater than those used by Nelson et al. The extracts were active in producing progestational changes in the rabbit uterus. Their extracts apparently had not been tested in this respect. As seen in table 6, only 1 animal (*136*) parturated late enough to show definite inhibition of parturition, and even in this case the effect was nothing like that obtained with gonadotropic preparations.

DISCUSSION

In considering the foregoing experiments and the work of others, it appears that gestation is prolonged because of a failure of the birth mechanism and not because of death of the fetuses. To support this, one need only point out that the young are term size and frequently much larger when parturition has been delayed. Live young have also been found as late as 25 days. Apparently pregnancy continues in a normal manner, without interference with fetal development and growth, up to about the time of parturition; then the placentae separate from the uterine wall, but the young are not expelled and die in the uterus. Resorption follows and may result in the death of the pregnant female.

The complete explanation of how gonadotropic hormones prevent parturition cannot be made at this time, but it seems reasonable to assume that it is not a result of a specific substance acting directly on the uterus. If such a substance existed, it would have to be present in FSH, LH, pregnant mare serum, PU, and unfractionated pituitary extracts. Furthermore it would have to be present in 12 mg. of APE with hemin when it is not present in 12 mg. of APE without hemin.

If one assumes the action to be indirect, the first structure to consider would be the ovary. As shown in table 2, 0.1 gm. equivalent of LH was effective when injections were begun on the 12th day, but did not affect parturition when begun on the 16th day. Therefore failure of parturition does not depend on the gonadotropic substance present just preceding and at parturition. If the gonadotropic substances act through the ovary, one would expect more time to be necessary than if the action were directly on the uterus.

It is interesting to note that the LH which had no effect in immature animals produced ovaries as large as 191 mg. in pregnant adults. One must conclude that the LH reacts with FSH from the rat. In the same manner, FSH must work with luteinizing substances of the rat itself in order to produce very large, solidly luteinized ovaries. Evans et al (13) report excessively luteinized ovaries and prolonged gestation in rats injected with antagonist.

Those gonadotropic extracts which best inhibit parturition produce a heavily luteinized mulberry ovary. These data suggest that progestin is the inhibiting factor. However, the data in this paper show that progestin in the amounts used does not inhibit parturition. Either the amounts administered (3 Rb.U. per day) were too small, or the extracts employed by Nelson et al contained something which is missing in the extracts used in these ex-

periments. The methyl alcohol extracts contained large amounts of solids and were probably toxic, so that any delay in parturition obtained could not be attributed to hormonal action.

The results obtained with smaller amounts of pregnant mare serum (0.1 c.c. and 0.2 c.c. per day) are suggestive of results obtained by D'Amour and Dumont (3) with estrin injections during the later part of gestation. They obtained abortion in some animals and prolonged gestation in others with a mixture of dead and live young parturated. However, the pregnant mare serum injected animals did not show vaginal cornification at any time during the injections. The author was unable to produce vaginal cornification or inhibit parturition in 2 rats injected with 40 R.U. of theelin per day from the 16th to the 22nd day of gestation. D'Amour and Dumont did not

TABLE 5. RATS TREATED WITH PROGESTIN

No. of Animals	Extract	Period of Injection	Amt. per Day	Result
3	A	16–22	0.5 Rb. U.	Parturated at term
3	B		0.5 Rb. U.	Parturated at term
2	B		3 Rb. U.	Parturated at term
2	B		1 Rb. U. plus 6 R.U. theelin	Parturated at term
2	B		1 Rb. U. plus 10 R.U. theelin	Parturated at term

A = Acid alcohol extract (16) prepared by H. L. Fevold, 45 gm. per Rb.U.
B = Prepared by R. K. Meyer, according to a modification of the Allen-Meyer method (17), 50 gm. per Rb.U.

state whether or not they obtained cornification when enough estrin was injected to interrupt or prolong gestation.

Since it has been definitely shown that progestin does not inhibit uterine motility and estrin does not sensitize the uterus to pitoccin in the rat (11), we need not look for the explanation of parturition or failure of parturition on this basis. However, Reynolds (12) has suggested a different explanation based on the influence of progestin and estrin upon uterine distention. He found that, under estrin stimulation, uterine tone resisted distention and under progestin the uterus was flaccid. He suggests that parturition occurs when estrin becomes dominant over progestin and the uterus is under optimum distention for the most efficient contractions. This again brings us to the expectation that sufficient amounts of progestin will inhibit parturition. If that can be demonstrated with purified extracts of corpus luteum tissue, a logical explanation of inhibited parturition by gonadotropic substances can be made.

Even if the sequence of events occurring at parturition is shown to result from changes in the estrin-progestin balance, one cannot look to the ovary alone as the source of these hormones. Numerous investigators have established the presence of progestin and estrin in the human placentae, and the work of Newton (14), Haterius (15), and others shows the placentae to have a definite influence on gestation in the rat. It seems that the gonadal hormones from the placentae, ovaries, or both, control the normal course of gestation and parturition, and any interference with their production results

TABLE 6. RATS TREATED WITH METHYL ALCOHOL EXTRACT OF CORPORA LUTEA

Animal	Extract	Period of Injection (Days)	Amt. per Day	Result
130	C	16–22	1.5 cc.	P[1] 9 live, 22 days
131	C		1.5 cc.	P 5 live, 23 days
133	D		1.25 cc.	P 5 live, 22½ days
134	D		1.25 cc.	P 3 live, 2 dead, 23 days
136	D		1.25 cc.	P 3 live, 3 dead, 23½ days

C = Methyl alcohol extract in aqueous suspension (9). 12 gm. per cc. 75 gm. per RB.U.
D = Methyl alcohol extract purified with benzene and acetone, injected in oil (9). 19 gm. per cc. 75 gm. per RB.U.
[1] Parturated.

in a deviation from the normal course of events. This interference with production of gonadal hormones can be effected by injections of gonadotropic substances. When this is done during the later part of gestation, parturition is inhibited. The exact means by which this occurs cannot be satisfactorily explained on the basis of research done up to the present.

SUMMARY

FSH and LH injected into pregnant rats during the later part of pregnancy inhibit parturition. Heavily luteinized, mulberry type of ovaries are produced by both extracts. The fetuses live up to term or beyond, and in some instances are abnormally large. Unfractionated pituitary extract inhibits parturition and produces heavily luteinized ovaries in the same manner as FSH or LH. A subminimal amount of unfractionated extract is able to inhibit parturition when hemin is added to it. Pregnant mare serum injected into pregnant rats produces ovaries which contain many large follicles as well as corpora lutea, and it is not as effective for inhibiting parturition as is unfractionated pituitary extract. Two different, highly purified preparations of progestin did not inhibit parturition even when 3 RB.U. per day were given from the 16th to the 22nd day. Methyl alcohol extracts of corpora lutea and methyl alcohol extracts of corpora lutea purified with benzene and acetone were unable definitely to inhibit parturition.

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THE INDUCTION OF BROODING BEHAVIOR IN THE JEWEL FISH[1]

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Many cichlid fishes, including the Jewel Fish, *Hemichromis bimaculatus,* brood their eggs and young. The eggs are guarded and fanned; the young are protected against the approach of other fish. For several days after hatching, the young which stray from the school are seized by either parent and spit out among the remainder of the school. Both parents 'call' their young by abrupt fin movements which are made at no other time (1). Unlike rats and mice (2), the Jewel Fish, which has raised one brood, readily distinguishes between its own young and those of other species, for it will collect and guard its own young but devour foreign species introduced at the same time (1).

Prolactin has been claimed to induce the brooding behavior of fowl (3) and maternal behavior in rats (4). The results obtained with the latter are by no means clear cut, and Leblond (2) has shown in mice that the "retrieving, licking and cuddling of the young, which may be considered the main features of maternal behavior, may be initiated and maintained without hormonal intermediaries." In the fowl, Ceni (5) found that an extract of several glands (chiefly thyroid and suprarenal would induce broodiness, but that blinding or even alcoholic poisoning would have the same effect. No attempt has been made previously to determine the hormones or other factors which may be responsible for the periodic occurrence of brooding behavior in fishes. In view of the primitive position of fishes in the vertebrate series, it has seemed desirable to secure evidence as to the nature of mechanisms inducing brooding in these forms.

METHODS

The Jewel Fish at temperatures of 80-84°F. spawns usually at intervals of from 7 to 10 days, provided the young are removed a short time after they hatch. The Jewel Fish employed in these tests were isolated in separate aquaria of 15 gal. capacity, and maintained together in a greenhouse at this temperature. Each fish was given a preliminary test to make sure that it was non-broody. Young Jewel Fish, 4 to 10 days of age (since hatching), were introduced into the tank. If the young were eaten the adult was considered non-broody; if they were spared, the adult was discarded. Non-broody adults were injected daily with the hormone or control substance. They were also tested daily with the young. If they failed to eat them and gave well-marked

[1] Supported by a grant from the Josiah Macy, Jr., Foundation.

'calling movements' of the fins, they were considered to be broody. If they tolerated the young, but failed to give the call movements, a further test was made. Young of the same size but of a different species of cichlid (*Cichlasoma bimaculatum* or *C. cutteri*) were introduced into their tank. If the adults ate these young, but still tolerated young of their own species, they were assumed to be broody. If the adults tolerated both kinds, it was assumed that the fish were in poor health and they were discarded. A non-broody Jewel Fish will eat eggs of its own species introduced into its tank. Since these were not always available the tests recorded below were made only with young fish.

Jewel Fish of both sexes were selected for the tests, and divided according to their histories into 3 groups: *a)*, fish which had both spawned and brooded, *b)*, fish which had spawned but never brooded, and *c)*, adults which had never spawned nor brooded. Part of each series was gonadectomized and allowed to heal before being tested. The injections were made intraperitoneally and included *a)*, prolactin in phenol,[2] *b)*, dry lactogenic hormone[3] dissolved in distilled water and Sorensen's pH 7.38 phosphate buffer solution, *c)*, corpus luteum extract, *d)*, corpus luteum extract followed by prolactin, *e)*, proluton, *f)*, antuitrin-S, *g)*, thyroxin, *h)*, amniotin, *i)*, anterior pituitary extract (Squibb), *j)*, post-pituitary extract (Squibb), *k)*, sheep whole gland and *l)*, fresh pituitaries from catfish and codfish. Control Jewel Fish were injected with *a)*, 0.1 per cent and 0.5 per cent solution of phenol, *b)*, 0.5 per cent solution of chloretone, *c)*, 0.1 per cent solution of sodium hydroxide, *d)*, 0.65 per cent saline solution. Additional fish were first fed desiccated thyroid, then immersed in 2.5 per cent alcohol for half-hour periods, or else subjected to total darkness for two or three weeks.

The dosages of the extracts and other substances varied with the size of the fish. It was found expedient to divide the fish arbitrarily into 3 groups according to their total length, large (10-11.5 cm.), medium (7.5-10 cm.) and small (5-7.5 cm.). The dosage was varied according to the group size the fish fell into, instead of according to the actual weight of each fish.

RESULTS

Prolactin. Table 1 shows the results obtained in the prolactin and phenol experiments. It was found that fish treated with prolactin could be induced to brood normally providing they had spawned at least once before the injections were started. Not a single fish without this experience responded, even though large doses were administered. Further, it will be noted that while 86.1 per cent of the experimentals, which had both spawned and reared young brooded, only 29.4 per cent of those with spawning experience alone were induced to care for young. The fact that any brooded at all is proof that a previous brooding experience is not a necessary antecedent for a successful result with prolactin treatment.

Control fish injected with phenol developed parental care in approximately half as many cases as those treated with prolactin, but the average

[2] We are indebted to Parke, Davis & Co. through the kindness of Dr. O. Kamm, and to E. R. Squibb & Sons through the courtesy of Dr. H. S. Newcomer for this material.
[3] Kindly supplied by Parke, Davis & Co.

dosage required was considerably greater. Hence the greater effect of prolactin should be attributed to the hormone and not the preservative. Phenol failed to induce brooding behavior in 6 out of 7 fish which had no previous brooding experience.

At constant temperature the breeding cycle in the Jewel Fish, as stated above, is very regular. If a fish was isolated from its eggs at the end of spawning, the dosage of prolactin required to produce parental care varied directly with the time elapsed since spawning (table 2). That is, a fish only 2 to 4 days removed from its eggs responded to injection much more quickly (8.5 B.U.) than one approaching the time for its subsequent spawning (6 to 9 days, 25.5 B.U.). However, once the normal breeding cycle of 7 to 10 days was exceeded (due to unknown conditions in particular fish), the dosage

TABLE 1. EFFECT OF PROLACTIN ON THE BROODING BEHAVIOR OF THE JEWEL FISH

		Normals					Castrates					Total		
		Cases	Broodings	Per cent of Broodings	Av. Total Dose of Broodings	Av. Total Dose of Failures	Cases	Broodings	Per cent of Broodings	Av. Total Dose of Broodings	Av. Total Dose of Failures	Cases	Broodings	Per cent of Broodings
Fish with spawning and brooding experience	Prolactin in Phenol	20 M	17	85.0	14.1 B.U.	48.3 B.U.	16 L	14	87.5	14.8 B.U.	35.0 B.U.	36	31	86.1
	Phenol- 0.1%	6	4	66.6	0.85 cc.	1.95 cc.	7	3	42.8	0.55 cc.	0.8 cc.	13	7	53.8
	Control 0.5%	7	3	42.8	0.50 cc.	0.81 cc.	1	1	100.0	0.8 cc.		8	4	50.0
	Saline 0.65%	23 M	2	8.6	3.8 cc.	4.35 cc.	3 L	0	0.0		5.8 cc.	26	2	7.7
Fish with spawning but no brooding experience	Prolactin in Phenol	12 SM	3	25.0	35.0 B.U.	45.1 B.U.	5 ML	2	40.0	22.0 B.U.	38.3 B.U.	17	5	29.4
	Phenol- Control 0.5%	6	0	0.0		0.79 cc.	1	1	100.0	0.8 cc.		7	1	14.2
	Saline 0.65%	6 S	0	0.0		2.6 cc.	2 M	0	0.0		2.6 cc.	8	0	0.0
Fish without spawning or brooding experience	Prolactin in Phenol	9 S	0	0.0		48.0 B.U.	11 SM	0	0.0		40.0 B.U.	20	0	0.0
	Prolactin in Phosphate	5 S	0	0.0		40.0 B.U.	5 S	0	0.0		40.0 B.U.	10	0	0.0

S = 5 - 7.5 cm. L = 10-11.5 cm.
M = 7.5-10 cm. 4 B.U. = .1 cc.

became variable and unpredictable (16 to 22 days, 19.2 B.U.). The length of time that a fish is isolated from its young also had a modifying effect. Recent brooding experience tended to lower the fish's threshold to prolactin.

Male Jewel Fish were found to respond to a lower dosage of prolactin than did females. This is correlated with the fact that the breeding cycle of the male is much more flexible than the female's, and his behavior is largely determined by such environmental conditions as the presence of a courting female (6). Castrated Jewel Fish required less prolactin and responded more frequently than did the intact fish. This is correlated with the fact that normal brooding is induced in the intact fish soon after the sex products are shed merely by the presence of young. Phenol-injected fish showed only slight sexual differences in response and no appreciable difference in the effective dosage for normals and castrates (table 1).

Finally, an attempt was made to induce mature fish with no previous

spawning experience to brood by first 'priming' with corpus-luteum extracts, and then treating with prolactin. Seven fish each received a total dosage of 0.5 cc. corpus luteum extract (Lilly) followed by 1 cc., or 40 B.U., of prolactin (Squibb). Three other fish were given 1 cc. of corpus luteum extract and then 1 cc. prolactin. None of these fish developed the brooding behavior as a result of the treatment.

Other preparations tested. The induction of brooding behavior in the Jewel Fish by prolactin treatment was apparently not due to the irritation caused by the injection. A series of Jewel Fish of both sexes was injected in exactly the same manner with a 0.65 per cent solution of sodium chloride. Only 2 out of 34 fish treated in this manner developed brooding behavior.

TABLE 2. INDUCTION OF BROODING. RELATION OF PROLACTIN DOSAGE TO STAGE IN THE BREEDING CYCLE

(Normal 7–10 days)

Fish removed from eggs for isolation	Cases	Number of Broodings	Average Total Dosage (B.U.)	Average Dose ♂♂	Average Dose ♀♀
Treatment started 2 to 4 days after last spawning	7	5	8.5	6.2	10.0
Treatment started 6 to 9 days after last spawning	5	4	25.5	25.0	26.2
Treatment started 16 to 22 days after last spawning	3	3	19.2	20.0	17.5
Fish removed from young for isolation. Treatment started 1 to 4 days after separation from young	5	5	7.5	8.8	2.5

The fact, however, that 2 did respond may be considered evidence that mechanical factors cannot be ruled out entirely.

Results secured with substances other than prolactin and its controls are listed in table 3. The very great success achieved with injections of corpus-luteum extract is striking. Every fish treated, which had a previous brooding record, began to brood normally. The influence of previous experience noted in the cases of prolactin-treated fish was repeated here. Only 20 per cent of those with spawning experience alone responded to injection, and fish without spawning or brooding records could not be made to care for young at all. However, sexual differences and castration had no discernible effect upon the quantities of corpus-luteum extract needed to bring about brooding behavior. Chloretone-injected controls failed to brood. Hence the effect of corpus-luteum extract is not to be attributed to its preservative.

Proluton treatment closely approximated the results with corpus-luteum extract, producing 90 per cent broodings in fish with previous brooding experience. Antuitrin-S was effective in 57 per cent of the cases but its control, phenol (0.1 per cent and 0.5 per cent), gave 52.4 per cent success. Similarly, thyroxin produced brooding in 25 per cent of the cases, and its sodium

TABLE 3. EFFECT OF VARIOUS TREATMENTS ON THE BROODING BEHAVIOR OF THE JEWEL FISH

Treatment	Fish which had previously reared young										Fish which have spawned but never brooded					Fish which have never spawned nor brooded									
	Normal					Castrate					Normal					Normal					Castrate				
	Cases	Broodings	Per cent	A.T.D.B.[1]	A.T.D.F.[2]	Cases	Broodings	Per cent	A.T. D.B.	A.T. D.F.	Cases	Broodings	Per cent	A.T. D.B.	A.T.D.F.	Cases	Broodings	Per cent	A.T. D.B.	A.T. D.F.	Cases	Broodings	Per cent	A.T. D.B.	A.T. D.F.
Corpus Luteum in Chloretone	9	9	100.0	0.61 cc.		4	4	100.0	.78 cc.		10	2	20.0	.9 cc.	1.3 cc.	13	0	0.0		.87 cc.	7	0	0.0		.94 cc.
Chloretone Control 0.5% Sol.	10	0	0.0		1.4 cc.	1	0	0.0		1.6 cc.															
Proluton in Oil	10[3]	9	90.0	0.66 cc.	1.0 cc.																				
Antuitrin-S in Phenol	11	5	45.4	0.15 cc.	0.26 cc.	3	3	100.0	.2 cc																
Anterior Pituitary—(Squibb)	6[4]	2	33.3	0.7 cc.	0.9 cc.																				
Post-Pituitary Extract (Squibb)	2	2	100.0	0.27 cc.																					
Sheep Whole Gland	2	2	100.0	0.3 cc.												9	0	0.0		.7 cc.					
Fresh Cat- and Codfish Pituitary	10[5]	3	30.0	10.2 pit.	10.2 pit.																2	0	0.0		24 pit.
Fresh Fish Optic Lobe—Control	4	1	25.0																		2	0	0.0		
Amniotin	10	0	0.0		7.2 cc.						4	0	0.0		3.8 cc.										
Testosterone Propionate	2	0	0.0		0.5 cc.																				
Thyroid-desiccated—Fed	10	2	20.0	9.5 gr.	11.5 gr.																				
Thyroxin 0.1% Sol.	20	5	25.0	0.4 cc.	0.91 cc.																				
NaOH Control	10	2	20.0	0.5 cc.	0.97 cc.																				
Bath Alcohol 2.5% ethyl	10	1	10.0	30 min.	63.2 min.						2	0	0.0		30.0 min.										
Darkness	10	0	0.0		2.6 wk.						2	0	0.0		3.0 wk.										

[1] A.T.D.B. = average total dose of broodings.
[2] A.T.D.F. = average total dose of failures.
[3] = 1 spawned during treatment.
[4] = 2 spawned during treatment.
[5] = 4 spawned during treatment.

hydroxide control in 20 per cent. Desiccated thyroid fed by mouth developed brooding in 2 out of 10 fish, or 20 per cent. Both thyroxin injection and thyroid feeding always produced a characteristic, pearly opalescence in the scales of the fish treated.

Two mature Jewel Fish, which had no previous spawning experience, were given 0.5 cc. of testosterone propionate[4] divided over a week's period. Both failed to show any brooding behavior.

Both anterior pituitary extract and fresh catfish and codfish pituitary injections showed moderate success, 33.3 per cent and 30 per cent, respectively. During the course of experimentation, however, it was discovered that 33.3 per cent of the treated fish spawned during anterior pituitary injection and 40 per cent after fish pituitary treatment. The stimulation of ovulation by the administration of anterior pituitary hormone has often been demonstrated in fish, amphibia, and other animals, and it is evident here that there was an even chance whether ovulation or brooding would be stimulated. Post-pituitary extract and sheep whole gland extract both gave brooding in the 2 cases of each tested. Fresh fish pituitary and sheep whole gland failed to induce brooding in fish without spawning experience. One fish out of 4 controls injected with fresh fish optic lobe substance brooded.

The effect of alcoholic poisoning and of darkness on the brooding behavior of the Jewel Fish was studied, since these have been stated to have some influence in the case of the domestic fowl (5). Darkness yielded no positive results and 1 fish out of 10 brooded after immersion in 2.5 per cent alcohol for one-half hour.

DISCUSSION

Brooding behavior cannot be elicited in the untreated cichlid out of the breeding period. If a Jewel Fish is nearly ready to spawn, the sight of young fish in an adjacent tank may sometimes induce the 'calling movements' of the fins (1). Although this has been observed only 2 or 3 times in more than 500 spawnings, the fact that it occurred at all is evidence that a spawning need not precede a normal brooding. Whether or not prolactin is actually released by a fish at the time of brooding is unknown, although it is apparently present in the pituitary, for Leblond and Noble (7) showed that catfish pituitaries will induce a typical epithelial hypertrophy when implanted over the crops of immature pigeons.

Since a wide variety of hormones will induce brooding behavior in the Jewel Fish it is possible that the changes taking place in several glands of the body at the time of spawning are actually responsible for the induction of brooding behavior in this form. It is nevertheless clear, from the results stated above, that both sexes of the Jewel Fish must go through one spawning before a maturation of the brooding mechanism of the central nervous system is accomplished. This mechanism is apparently located partly in the corpus striatum, since lesions of this area permanently distort the pattern of brooding behavior (8).

[4] We are indebted to Ciba Pharmaceutical Products, Inc. through the kindness of Dr. R. MacBrayer for this material.

Ceni (5) found that partial castration, blinding (even if merely effected by suturing the eyelids) and alcoholic poisoning would promote the development of brooding behavior in the fowl. It would seem that any agent which tended to depress gonad function might elicit brooding behavior. Further, Ceni (5) found that his pluriglandular extract, which was very effective in promoting brooding, inhibited sexual functions, but in the case of the male did not stop them entirely. This extract consisted of thyroid 0.1 gr., suprarenal 0.09 gr., hypophysis 0.025 gr. and parathyroid 0.005 gr. A unit dose of 0.22 gr. per 1.5 kg. of fowl, if injected daily for 3 or 4 months, would give a maximum brooding response and yet not prevent sexual functions. In the experiments with fish, reported above, brooding behavior was induced in completely castrate fish by corpus luteum extracts. Hence it would seem that hormonal extracts in fowl and fish act directly on the nervous system to induce brooding behavior instead of merely depressing gonad function.

SUMMARY

Brooding behavior in the cichlid, *Hemichromis bimaculatus,* with spawning experience can be produced experimentally by treatment with a number of substances. Most effective, in order, when a large series of fish is tested are corpus luteum, proluton, and prolactin. Anterior pituitary extract, fresh fish pituitary, thyroxin, desiccated thyroid and phenol in 0.1 or 0.5 per cent solution are effective to a much less extent. Sexually mature fish without spawning experience cannot be induced to brood with any of the above substances. Previous brooding experience is not essential but greatly increases the response following treatment. At the beginning of their breeding cycle fish require less prolactin than those about to spawn; castrates require less than normals; and males have a lower threshold to prolactin than do females.

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CASE REPORTS

PITUITARY DWARFISM

CASE REPORT ILLUSTRATING RESPONSE TO TREATMENT[1]

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Since the publication of Engelbach's contribution on 'The Growth Hormone' in 1932 (1), a number of articles concerning its clinical effects have appeared in the literature (2, 3, 4, 5). However, the total number of patients treated remains small, and the results of treatment are variable. Therefore a report of the results of treatment of a single patient seems justified. The age of the patient when treatment was begun and the sexual development during treatment are worth recording.

CASE REPORT

A white schoolboy was admitted to the Endocrine Clinic, in June 1934 because of lack of growth and sexual development. His mother stated that he was of normal length and weight at birth, but that even then the genetalia were conspicuously small. There is no history of severe illness during childhood, except mastoiditis requiring operation at the age of 8 years. He had had no illness for 5 years before admission, but had been treated in the Children's Clinic of the Dispensary since February, 1932, for malnutrition. We are indebted to that clinic for his earlier measurements.

In April, 1934, the patient was admitted to the Diagnostic Ward of the Dispensary for study. He was then 16 years 4 months old, 59¾ inches tall and weighed 87 lb. Physical examination showed a slender, ill-nourished, rather apathetic boy, with eunuchoid figure and smooth 'girlish' skin. There was no growth of body hair except a few fine hairs in the pubic region. He had never shaved. The penis was infantile, the testicles pea-sized (about 1 cm. in diameter). The arms and legs were disproportionately long, the fingers long and tapering. His voice was high-pitched and 'thin.' There was marked lordosis. No evidence of organic disease was found. The I.Q., on age basis, was 73. The neurological opinion was that the patient had a congenital mental and physical inferiority, with little chance of normal development.

Laboratory Findings. The blood showed a hemoglobin of 90 per cent (Sahli), red count of 4,740,000, and white count 8,650; differential count showed 43 per cent polynuclears, 43 per cent lymphocytes, 7 per cent monocytes, 4 per cent eosinophiles, 2 per cent basophiles, and 1 per cent band forms. The red cells appeared normal. The Hinton, Wassermann and Kahn Tests showed negative findings. Sedimentation rate (Westergren) was: 20 minutes, 3 mm.; one hour, 10 mm.; two

[1] The study was aided in part by a grant from the Charlton Research Fund, Tufts College Medical School.

hours, 20 mm., the blood calcium was 11.9 mg. per 100 cc., cholesterol 100 mg., uric acid 2.7 mg., and non-protein-nitrogen 31 mg. per 100 cc. One single urine specimen and two 24-hour specimens showed the faintest possible trace of albumin, but were normal in all other respects. A glucose tolerance test with 100 gm. of glucose gave the following results: fasting blood sugar 134 mg.; ½ hr. 238 mg.; 1 hr. 225 mg.; 1½ hr. 179 mg.; 2 hr. 143 mg.; and 2½ hr. 126 mg. per 100 cc. There was no glycosuria during the test. The visual fields and retinae were normal. Roentgenograms of the skull gave no evidence of pituitary or cerebral pathology. The basal metabolic rate on December 15, 1933 was —27.5 per cent. On January 10, 1934, it was reported to be —6. There was no change in the patient's condition noted to explain the discrepancy between the two observations, and the lower rate was more consistent with his appearance and symptoms.

Treatment was begun in the Endocrine Clinic in June, 1934. The preparations

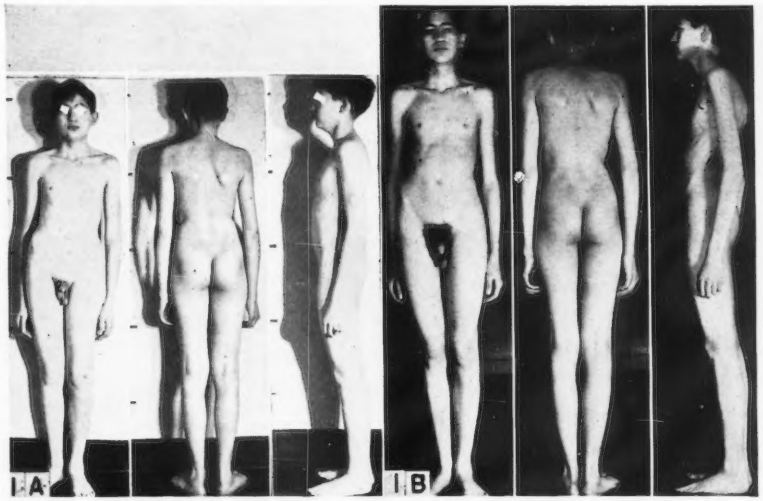

Fig. 1. Anterior, posterior and lateral views of patient at beginning (*A*), and at end (*B*) of treatment.

used, the amounts administered, the increase in height during treatment as compared with Engelbach's minimal normal prediction, and the appearance of various developmental criteria are shown in figure 2. The changes in other body measurements are shown in table 1. The patient's physical appearance at the beginning and end of treatment are shown in figure 1, *A* and *B*.

The treatment with 'glypitan'[2] was tried before the patient's admission to the Endocrine Clinic, because it was impossible at the time for him to come in regularly for hypodermic medication. The notes on his record during that period do not indicate any response to the medication.

As soon as arrangements could be completed for his regular attendance at the Endocrine Clinic, treatment with antuitrin-S and antuitrin-G was begun.[2] The dosage at first was 1 cc. of each preparation 3 times weekly. These amounts were increased to 2 cc. as the patient's tolerance was established. The first indication of

[2] The antuitrin-S and antuitrin-G used were generously supplied by Parke, Davis & Company. The glypitan was supplied by Schieffelin & Company, and the orchic extract by Colwell Pharmacal Corporation.

TABLE 1. DETAILED BODY MEASUREMENTS AT THE BEGINNING AND NEAR THE END OF TREATMENT

Body measurements (inches)	February 1935	November 1936
Arm length, coracoid process to index finger tip	28.5	30
Anterior sup. spine to ground	37	39.75
Total span of arms	64	69
Upper arm	11.5	13.5
Upper leg, ant. sup. spine to int. mal.	18.5	21
Lower leg, int. mal. to tip of tibia	18	18
Height	62.5	66.75
Weight	92.5 lb.	101.75 lb.
Testicular measurements		
Right	2.5 cm.×1.5 cm.	4 cm.×2 cm.
Left	2 cm.×1 cm.	4.5 cm.×2.5 cm.

response was an increase in growth of pubic hair, noted about 3 weeks after treatment was begun. From that time on the response to treatment was, as the figure 2 shows, interrupted only once. The cause of that interruption is somewhat speculative, but its coincidence with the administration of some Orchic extract[2] suggests at least that the latter had an inhibiting effect upon growth, either by depressing the function of the patient's own pituitary or by reducing the responsiveness of his

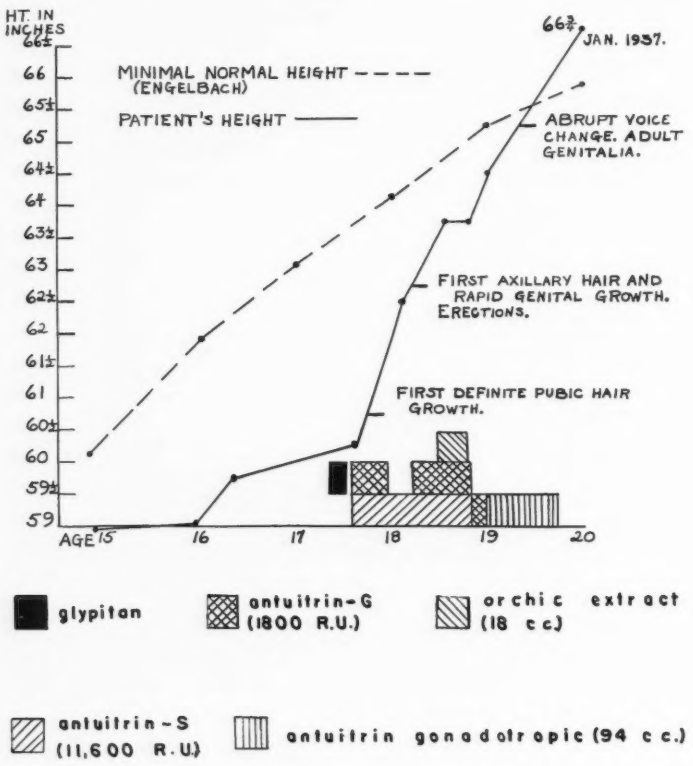

FIG. 2.

effector tissues to the growth extract. The evidence on this point is as yet too debatable to discuss in a brief report.

With the single interruption just referred to, the gain in height at all times continued to exceed the minimal normal gains as predicted by Engleback (1). It seems hardly probable that such a gain would have been initiated spontaneously at the age of 17 years and 8 months, or would have continued through the twentieth year in a patient whose growth during the preceding 2½ years had been so conspicuously below prediction. At no time during treatment was there any evidence of disturbance of carbohydrate metabolism, a possibility which has been indicated by the work of Evans, Myer, Simpson and Reichert (2). These investigators administered growth hormone to dogs and produced in two animals hyperglycemia, glycosuria and loss of weight. In two other dogs identically treated, no effect on carbohydrate metabolism was noted, and the authors conclude that the results could be explained only on the basis of 'constitutional factors'. The necessity of watching the carbohydrate metabolism during treatment with growth hormone is, however, apparent.

One other result of treatment is worthy of comment—the psychological effect of the growth and development. We have called attention in a previous paper (6) to the psychic trauma which results from a sense of personal peculiarity, and this patient's personality at the beginning of treatment was typically asocial and introverted. With the development of normal secondary sex characteristics and normal stature there was a marked change of attitude; a desire for companions of both sexes replaced former recluse habits, and he became happier and more alert. We believe these changes are due only indirectly to hormonic factors, but are the direct result of a comforting sense of normality. Whatever its cause, this psychological effect furnishes a considerable argument for beginning treatment promptly when growth or developmental deficit is obvious.

SUMMARY

A case is reported illustrating the effect of treatment in a boy 17 years and 4 months old, exhibiting sexual infantalism and dwarfism of the eunuchoid type. An increase of 6½ inches in height, and normal sexual development, occurred during the period of treatment. The reasons for ascribing these results to treatment rather than to spontaneous growth and development are briefly stated.

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CLINICAL AND LABORATORY NOTES

THE TREATMENT OF A CRETIN WITH ANTERIOR PITUITARY EXTRACT

G. DE M. RUDOLF

Born in 1930, the patient was first seen by Dr. R. H. Allport of Guildford at the age of 18 months. He was then fat, expressionless and unable to sit up. He had the mental development of a child of 3 months, and was obviously suffering from thyroid deficiency. He improved rapidly on ¼ gr. thyroid extract daily and, although young for his age, became otherwise apparently normal.

At 3½ years of age he went to Egypt, and while there was operated on for mastoiditis. Following the operation, improvement ceased. He became more dull on thyroid extract. When 6 years old he returned to England and, although thyroid extract was not given, began to improve. Thyroid was then ordered, ¼ gr. daily, but after one dose he became dull, lethargic and drowsy for an entire day.

The author first saw the boy at this time. He was then a typical cretin, the diagnosis being confirmed by Dr. R. M. Stewart. The peculiar color of the skin, the supraclavicular pads of fat, the tongue, the indistinct speech, the protuberant abdomen, the depressed bridge of the nose and the mental retardation (under 3 years) were all present. The heart and lungs were normal, but the liver was enlarged to about ½ in. below the costal margin. He was short, 40⅝ in., and below average weight, viz., 39½ lb.

Thyroid extract (siccum), 1/10 gr. daily, was given for 1 week. He became appreciably duller and less active. Two months later he had gained 4 lb. in weight, but the height remained stationary.

As replacement therapy with thyroid extract had made the patient more dull on two occasions, an attempt was made to increase the stature with APE. Antuitrin-G, containing 10 R.U. per cc., was given subcutaneously in doses of from 1 cc. to 3 cc. on 5 occasions, at intervals of 4 to 7 days. After the 4th dose, which was 3 cc., the thyroid gland was noticeably enlarging, and the patient was becoming dull. These changes became very marked after the 5th dose, which was also of 3 cc., so the treatment was discontinued.

The Director of the laboratory in which the antuitrin-G had been made stated that the process used in its manufacture destroyed the thyrotropic principle, and that doses of 6 and 12 cc. given to young guinea-pigs over 3 days showed the absence of thyrotropic activity.

One month after the treatment described above had been stopped, the patient was less dull, and antuitrin-G was recommenced in doses of 1 cc. at fortnightly intervals for 6 weeks. The boy became markedly brighter. The treatment was omitted for 2 months. Dullness and apathy again occurred.

Antuitrin-G was recommenced and, after 3 doses of 1 cc. at fortnightly intervals, was given once a week in doses of from 1.5 to 2 cc. for 5 months. During this period an increasing brightness was observed. The patient became more alert and active and took a greater interest in his baby brother and his surroundings.

He now recalled spontaneously events which had occurred in Egypt 3 years before. He could read letters and short words, and could count. Although still backward (e.g., he could not recognise all colors) he was considered sufficiently advanced to attend school with normal children. Two months after this last course of treatment was begun, the skin began to become softer and less dry, the hair lost its dandruff and the face began to lose the yellow color of the cretin. The bridge of the nose developed. Within 1 month of the commencement, a gain of ¼ in. in

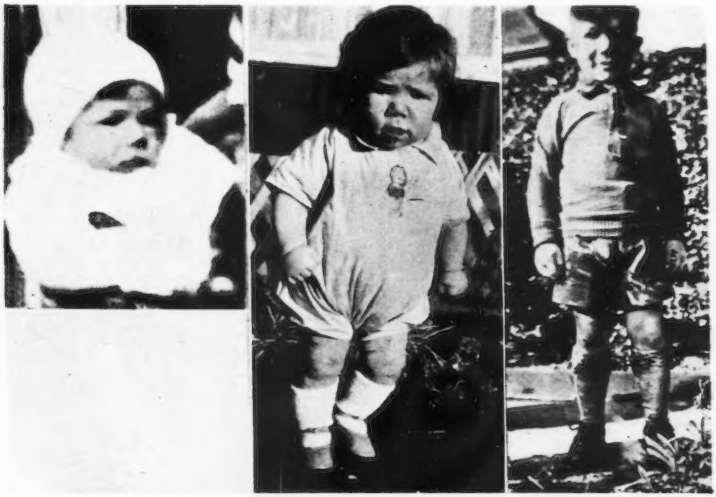

Fig. 1. *A*, Patient at 5 months; *B*, at 15 months; *C*, at 7 years.

height occurred, and similar gains were observed in the following month and in the last 2 months. The arms lengthened to the normal proportion; the weight remained stationary, around 45 lb., but the obesity decreased.

Unfortunately, whooping-cough developed, pneumonia followed and death ensued.

Figure 1 shows the patient at the age of 5 months, before the cretinism was apparent; at 15 months, when it had developed and at 7 years, after treatment had been given. The presence of the bridge of the nose in figure 1*A* and 1*C*, and its depression in 1*B* are clear. The relative increase in the length of the arms and the change of the fat baby legs to normal, but short, limbs can also be seen.

SUMMARY

A cretin who imroved on thyroid extract, ¼ gr. daily, ceased improving after an operation for mastoiditis and remained stationary. When given thyroid extract, ¼ gr. once, 1/10 gr. daily for 7 days and stimulation to enlargement of the thyroid gland by means of APE, he became, on each occasion, apathetic and more dull. Three doses of 1 cc. of antuitrin-G at fortnightly intervals were accompanied by improvement, but he became duller again after their cessation. Finally, doses of from 1 cc. to 2 cc. of antuitrin-G at fortnightly and weekly intervals for

5 months were accompanied by a steady improvement both physically and mentally.

My thanks are due to Dr. R. M. Stewart for kindly observing the case for me, to Dr. R. H. Allport for details of the early history and to Parke, Davis and Company for information about their product used in the treatment.

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UNUSUAL RESULTS FOLLOWING THE INJECTION OF EPINEPHRIN

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This paper reports some unusual results following the subcutaneous injection of epinephrin. The subjects were male and female general paralytics. No anaesthetics

TABLE 1

No.	Epinephrin in mg.	Systolic pressure at injection	Alterations in systolic pressure				Days since previous injection
			Max. rise in mm.	Percentage Rise	Max. fall in mm.	Percentage Fall	
1	.59	97	15	15.5	0	0	—
	.59	119	5	4.2	29	24.4	36
2	.59	149	4	2.7	40	26.8	—
	.59	143	0	0	53	36.9	13
	.59	163	0	0	75	46.0	10
3	.44	82	25	30.5	5	6.1	—
	.44	118	26	22	8	6.76	12
	.44	110	14	12.7	18	16.3	9
4	.29	94	13	13.8	15	15.9	—
	.29	104	2	1.9	14	13.5	7
	.29	93	1	1.1	25	26.9	20
5	.29	122	5	4.1	14	11.5	—
	.29	124	0	0	21	16.9	7
6	.29	112	24	21.4	13	11.6	—
	.29	102	17	16.7	6	5.9	7
7	1.18	110	0	0	12	10.9	—
	.59	118	6	5.08	22	18.6	12
8	.59	81	35	43.2	11	13.6	—
	.59	99	0	0	21	21.2	9
9	.29	97	5	5.16	13	13.4	—
	.44	96	6	6.25	14	14.6	12

were used. This is important, as many animal experiments have been vitiated by the use of anaesthetics. Wyman and Lutz[1] found that, in cats, small doses of ether increased the pressor responses to adrenalin. The responses become progressively

[1] Whyman, L. C., and B. R. Lutz: J. Physiol. 73:254. 1925.

greater up to one hour after the ether had been stopped. Large doses of ether had the opposite effect, decreasing the pressor response. Macdonald and Schlapp[2] found that ether and urethane produced depressor effects. Vincent and Curtis[3] noticed the same effects with chloroform and chloralose.

Subcutaneous injection is more suitable than intravenous, as temporary emotional changes affect less seriously a curve of 2 or 3 hours' duration than one lasting for less than 30 minutes. As the blood vessels contract, no alteration of the systolic pressure occurs immediately. In dogs, Luckhardt and Koppanyi[4] found that, with massage of the site of injection, the first change of pressure was noticed about 10 minutes after the injection. In the present experiments, the post-injection observations of the systolic pressure were begun from 3 to 15 minutes after the injections, and continued for from 120 to 220 minutes. From the beginning to the end of the experiments, the epinephrin was therefore being absorbed in a natural manner from the subcutaneous tissues. The injections were given at intervals of from 7 to 36 days.

Table 1 gives the maximum rises and falls of systolic pressure and shows the percentage alterations, based on the estimated levels at the time of injection. The pressure immediately before, at the time of, or immediately after an injection is of little or no value, owing to the effect of the emotions upon the readings. The pressure was taken before the injection, and at the time of injection it was estimated with the help of the first reading taken after the injection. The constant decrease of the maximum falls with injections subsequent to the first, in subjects receiving the same doses of epinephrin at each injection, suggest that this method of estimating the systolic pressure at the time of injection is reasonably accurate.

Summary. Changes in pressure consisted of pressor and depressor effects. A decrease of the pressor effect and an increase of the depressor effect were produced by the previous injection of from 0.29 to 0.59 mg. epinephrin. The decrease occurred in all of 10 experiments in 7 subjects. The increase occurred in 8 of 10 experiments in 7 subjects. The 2 experiments in which the increase did not occur were those in which small doses of 0.29 mg. were used. In 1 subject who received 1.18 mg. the decrease of the pressor action was not observed, but the increase of the depressor effect was recorded. At the second injection, an increase of dose of 50 per cent in 1 subject increased both the pressor and depressor effects. In subjects who received equal doses at each injection, a tendency occurred for the duration of the pressor effect to be diminished, and that of the depressor increased, with injections after the first. The altered effects were found from 7 to 36 days after previous injections. The effects of injections are cumulative. Greater effects were not observed when the intervals between injections were longer than 7 days.

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HYPOPHYSIS IN PROTEIN METABOLISM. IS THE PITUITARY FACTOR ACTIVE IN PROTEIN METABOLISM IDENTICAL WITH THE GROWTH HORMONE?

KARL E. PASCHKIS*

It has been reported in a previous paper that regulation of the amino acid-metabolism exists in normal persons, as can be proved by double administration of amino acid (gelatine) solution.[1]

If 50 gm. of gelatine (in 500 cc. water) is given to a normal fasting person, and the same test drink is repeated 3 hours later, the blood-amino acid level rises markedly after the first administration, whereas the second does not produce any rise at all or, at the most, only a very low one. This proves that there is a regulation of the amino acid-metabolism, probably due to nervous and hormonal influence, as discussed in the above-mentioned paper. In a further paper[2] we have proved that the pituitary anterior lobe has some very important influence on the regulation of amino acid-metabolism. In 3 cases of pituitary insufficiency (pituitary cachexia), the functional test, as described above, was abnormal, i.e., the aminoacid rise in the blood after the second administration of gelatine was as high as, or even higher than, the first. A factor responsible for the normal regulation is apparently missing in these cases. It is highly probable that a hormone of the pituitary anterior lobe regulates the protein metabolism. The pathologic result of our 'double test' in cases of pituitary cachexia would then be due to an insufficient output of this protein-metabolism hormone.

According to previous investigations[3, 4] and our own experimental results[2] there is a close relation between the pituitary growth hormone and the protein metabolic effect. Administration of growth hormone fractions of the pituitary anterior lobe, prepared according to Evans, produces a drop in the residual nitrogen substances of the blood (residual-N, urea-N, amino-N) in dogs, rats and guinea-pigs.

We have raised the question whether the pituitary substance active in protein metabolism is identical with the pituitary growth hormone, or whether there are 2 hormones, the growth hormone and the protein metabolism hormone, precipitated together when fractioning the pituitary extracts. We can try to answer this question by means of experimental research and by clinical observation. We have already published a single clinical observation, which does not seem to be in accordance with the assumption that growth hormone and protein metabolism hormone are identical: in one case of acromegaly our double test gave a pathologic result, i.e., a marked rise of the blood-amino-N-level after the second administration of gelatine. In cases of acromegaly we are certainly entitled to assume an increased output of growth hormone, whereas our test showed the same features as we had found in pituitary deficiency. In the light of this observation, doubts as to the identity of the two hormones in question seem reasonable.

But that this evidence is inconclusive is indisputable. A long standing case of

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acromegaly need not necessarily involve an increased output of growth hormone at the time of our investigation. It has not been tested in our case. It is possible that in former days there was an increased production of growth hormone with acromegaly developing in consequence. The acromegalic symptoms, not being to any large extent reversible, would persist and be present at a time when the production and output of growth hormone might have ceased, or might even be replaced by a diminished production due to exhaustion of the pituitary anterior lobe.

It is obvious that such theoretical speculations cannot solve the problem. I should like to emphasize that the reaction in the above-mentioned case of acromegaly is in no way typical of this disease. We have since had the opportunity to investigate another case of typical acromegaly: the result of the 'double test' was in this case normal.

Investigations in cases of pituitary nanosomia seemed to offer an opportunity to solve the question. Pituitary dwarfism is based upon permanent lack of pituitary

TABLE 1

	Amino-N (mg. %)						First Rise		Second Rise	
	Before	1 hr.	2 hr.	3 hr.	1 hr.	3 hr.	mg.	%	mg.	%
	After first administration				After second administration					
Case 1. *A.P.* No. 456	7.7	10.6	11.2	10.5	11.8	12.2	3.5	45	1.6	16
Case 2. *M.K.* No. 457	5.8	9.3	10.3	10.5	10.8	11.6	4.7	81	1.1	10

growth hormone. If the effect of the pituitary anterior lobe in protein metabolism were due to the growth hormone proper, a protein metabolism test would necessarily prove a lack of protein metabolic effect. For this reason, cases of pituitary nanosomia are more apt to solve the question of identity of growth hormone and protein metabolism hormone than does their counterpart pituitary gigantism or acromegaly. First, because we still cannot by our method test a surplus of protein metabolic effect with any certainty. In cases of pituitary gigantism or acromegaly we would expect a surplus of protein-metabolism hormone under the assumption of an identity between growth hormone and the factor active in protein metabolism, whereas the lack of protein metabolic effect to be expected in cases of pituitary nanosomia under the same assumption can easily be tested by our method. Second, as mentioned above, acromegaly may be the irreversible effect of a temporary surplus in production of growth hormone, whereas pituitary nanosomia is in all probability due to a permanent lack of growth hormone.

Thus, when studying cases of pituitary dwarfism we are always testing in a period of pathologic conditions of the hormone output, whereas in cases of acromegaly this may or may not be true.

As can be seen in table 1, the 'regulation effect' after double administration of gelatine is normal in both cases of pituitary nanosomia. We have shown previously that the 'regulation effect' is missing in cases of pituitary cachexia. Obviously, the factor responsible for the 'regulation effect' cannot be identical with the growth hormone. As on the other hand pituitary extracts containing growth hormone are active in protein metabolism[3, 4, 5] we may further expect that in these extracts, too,

[5] Teel, H. M., and Olive Watkins: Am. J. Physiol. 89:662. 1929.

the factor active in protein metabolism is not identical with the growth hormone but merely extracted together with it. This latter conclusion stands only under the assumption that the factor responsible for the normal 'regulation effect' is identical with the factor present in alkaline pituitary extracts producing a drop of blood nitrogen values in animal experiment.

In view of our experimental findings, it is our conception that a hormone of the pituitary anterior lobe is necessary in anabolic processes, in the utilization of protein metabolites and in the building up of body protein (protein metabolism hormone). It is present in alkaline pituitary extracts, and has until now not been isolated from growth hormone. The presence of the protein metabolism hormone in pituitary extracts can be tested by a fall of blood-amino and blood-urea-N-values after injection of the extracts to rats, dogs and guinea-pigs. A protein meal stimulates the pituitary anterior lobe and produces an increased output of protein-metabolism hormone. A second administration of protein, a few hours after the first one, leads only to a small rise of blood amino-N- or none at all, because there is plenty of protein-metabolism hormone at disposal, in consequence of the first protein administration. We have called this phenomenon 'regulation effect.' It is lacking in cases of pituitary cachexia, as here the first protein administration does not mobilize the output of protein metabolism hormone, either because the anterior pituitary lobe is destroyed, or because there is a functional injury preventing the normal response to the adequate stimulus. In consequence, the rise of blood-amino nitrogen after the second protein administration is very high, since the lack of the specific hormone prevents the anabolic process from starting promptly. The normal reaction of pituitary dwarfs proves that the effect is independent of the growth hormone; in other words, the protein metabolism hormone is not identical with the growth hormone.

Summary. The existence of a protein metabolism hormone of the pituitary anterior lobe can be assumed. It lowers the blood amino-N and the blood urea-N level of rats, dogs and guinea-pigs after administration of alkaline pituitary extracts. It prevents the rise of blood-amino acids if, 3 hours after a first administration of 50 gm. of gelatine a second administration of the same quantity follows. We call this phenomenon the 'regulation effect' in protein metabolism. In 3 cases of Simmonds's pituitary cachexia this regulation effect was missing. In 2 cases of pituitary nanosomia the 'regulation effect' was normal. This is considered proof that the protein metabolism hormone is not identical with the growth hormone. 'Regulation effect' was missing in one case of acromegaly previously published, and normal in a new case of acromegaly.

CURRENT ENDOCRINE LITERATURE

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ADVANCE ABSTRACTS

Hormone and electrolyte studies of patients with hyperadrenocortical syndrome (Cushing's syndrome). Anderson, Evelyn, W. Haymaker and M. Joseph. To appear in this Journal.

Benzene extracts of the blood of 3 patients with Cushing's syndrome prolonged the lives of adrenalectomized rats beyond the survival period of untreated controls. The 'cortin-effect' of 1 cc. of blood was equivalent to 4-6 gm. of fresh adrenal tissue. The extract of urine of one of these patients showed a similar effect. It was estimated that a 24-hour urine specimen contained the equivalent of 400 gm. of fresh adrenal cortical tissue. The sodium and potassium levels in the serum and the urinary excretion of these electrolytes in patients with Cushing's syndrome showed a shift from the normal which is diametrically opposite to that found in adrenal insufficiency. These studies would seem to indicate that Cushing's syndrome is produced by an excess of adrenal cortical hormone (cortin).

The effect of estrin on the gonad-stimulating complex of the anterior pituitary of parabiotic rats. Bunster, E., and R. K. Meyer. To appear in this Journal.

Estrin, as found in amniotin, was studied for its effect on the gonad-stimulating hormone of the anterior pituitary gland of castrated immature male and female rats in parabiosis with immature females. The data indicate that the pituitary of the castrated male develops a moderately higher gonad-stimulating activity than that of the female. There is no qualitative but only a quantitative difference in the sensitivity of the pituitary gland of the two sexes to estrin. The average daily amount of estrin in the form of amniotin necessary to prevent the increase of the gonadotropic hormone in the castrated male, 21 to 35 days old, is 1 R.U., and that necessary to produce the same result in the spayed female is 1/8 R.U.

The effect of antuitrin-S on the thymus of the young albino rat. Butcher, E. O., and E. C. Persike, Jr. To appear in this Journal.

Administration of 20 R.U. of antuitrin-S daily causes the thymus of the young albino rat to stop growing after 3 or 4 injections. Little change then occurs in the thymus with continued injections. Such an arrested growth cannot be induced before the rat is 17 or 18 days old. This arrested condition has persisted with continued daily treatment in the males for the duration of our experiments, which was 65 days. In the female a decline in the effectiveness of the hormone has occurred by the 30th daily injection. Upon cessation of injections, the thymus hypertrophies rapidly. The effect of antuitrin-S is through the gonads, since the hormone is ineffective in gonadectomized animals.

A method of assaying the potency of anterior pituitary extracts which increase liver fat. Campbell, J. To appear in this Journal.

A test is described whereby the activity of anterior pituitary extracts in increasing liver fat may be determined. Normal mice were fasted for 7 hours following the injection of extract,

and liver fat was then determined. The increase produced by the unknown extract was compared with that produced by a standard extract. The extent of the variations in the test have been estimated and certain variable factors considered. Among these it may be noted that females were more sensitive than males by the ratio 1.4 to 1. As the temperature was lowered, no decrease in the effect was observed, but rather a tendency for the effect to increase. An increase in liver glycogen accompanied the increase in liver fat. Under the same conditions as used for the liver fat test, mice may be used to determine the 'glycostatic effect' of anterior pituitary extracts and, after a preliminary fast of 6 hours, the 'glycotropic effect'. Under the same conditions also the resistance to the convulsions produced by insulin by the anterior pituitary may be studied. This resistance may increase sevenfold.

Insulin therapy in mental diseases. Conwell, D. V., and C. J. Kurth. To appear in this Journal.

Fifty-four mental patients given Sakel's insulin shock therapy were observed for the effect on the mental conditions, for a more standard way of administering the insulin, for means of recognizing impending danger and for untoward systemic and endocrine changes. The therapy was of distinct value in schizophrenia, in the manic phase of manic-depressive psychosis and in psychasthenia. It was of questionable value in the depressed phase of manic-depressive psychosis and in melancholia. The only laboratory test of value was the blood-sugar determination. About 3,500 capillary blood-sugar readings were made on these 54 patients. The preliminary blood-sugar curves or fasting levels were of no value in predicting the results, but they were of definite value in the pretreatment consideration of the patient's insulin tolerance. Daily blood-sugar readings made 5 hours after the insulin was given were helpful in managing the therapy and in avoiding danger. After the capillary blood-sugar reading reached the shock level of the 50-mg. vicinity a comparative 24-hour drop of 20 mg. or more presaged danger. Less insulin was required and part of the danger element was excluded by controlling the diets. Before we recognized these means of avoiding danger we had observed amentia, peripheral neuritis, serial shocks and gastro-intestinal hemorrhages. We observed no immediate or late changes in the parathyroid or pituitary gland functions. Transitory pancreas and gonodal hypofunction was not uncommon. Instances of decreased adrenal and thyroid gland functions were quite common and prolonged.

The effects of estrogenic hormone on the sex cycles of normal rats. Donahue, J. To appear in this Journal.

Normal, 66-day old female rats, injected with 500 I.U. of theelin daily for 27 days, were caused to enter into a diestrous period lasting for about 18 days after which estrus again appeared. Corpora lutea were caused to persist in rats of the same age injected with 200, 100, and 50 I.U. respectively each day for 10 days. Functional corpora lutea were not caused to persist when the dosage level was reduced to 25 or 10 I.U. daily for a ten-day period. It was concluded that estrogenic hormone, administered in daily dosages of 50 I.U. or above causes the release of whatever LH is present in the hypophysis as stored material. In all probability an actual stimulation of LH production in the hypophysis is not accomplished.

The hyaline change in the basophil cells of the pituitary body not associated with basophilism. Ecker, A. D. To appear in this Journal.

The hyaline change of Crooke was present to a marked degree in the basophil cells of the anterior lobe in 8 of 721 pituitary bodies (an incidence of 1.1%). The same change was present to a considerable degree in the cells of 4 of 72 basophil adenomas (an incidence of 5.5%). In none of the cases of this series in which the hyaline change was found had there been any clinical or postmortem evidence of pituitary basophilism.

Urinary thyrotropic hormone. Emerson, K., Jr., and W. C. Cutting. To appear in this Journal.

Urinary thyrotropic activity could not be demonstrated in normal man or normal animals, and only rarely in human hyperthyroidism. Transient urinary thyrotropic activity regularly followed thyroidectomy in animals and man, but could only rarely be demonstrated in spontaneous hypothyroidism.

Insulin-insensitivity: its possible relation to the pituitary gland. Flaum, G. To appear in this Journal.

Three groups of patients were studied by means of the glucose tolerance and simultaneous

glucose-and-insulin blood sugar curves. The 'insulin-sensitive' diabetics resemble normal individuals in showing a suppression of the post-glucose hyperglycemia in the glucose-and-insulin curves. In two patients with acromegaly and diabetes the simultaneous intravenous injection of insulin had little effect in suppressing the postglucose hyperglycemia. It is suggested that the tumor of the pituitary gland demonstrated in these patients is an etiologic factor in the 'insulin-insensitivity' exhibited by these patients.

Endocrine theories of dysmenorrhea. Fluhmann, C. F.

A series of 85 tests for estrogenic substances in the blood was conducted on 19 patients with primary dysmenorrhea. In 18 instances the results failed to show any variation from the normal. There are serious objections to accepting any of the theories which seek to explain the cause of primary dysmenorrhea solely as a deficiency or excess of either estrogen or progestin.

Diabetes mellitus associated with hyperthyroidism. Foster, D. P., and W. L. Lowrie. To appear in this Journal.

A group of 42 thyrodiabetics were studied. A total of 45 thyroidectomies were done. Cases were gleaned from 1616 diabetics and 1607 patients who had thyroidectomy. The incidence of thyrodiabetics to diabetic admissions was 2.41 per cent, and of hyperthyroid admissions 2.43 per cent. The highest incidence occurred in patients 51 to 70 years of age. Females predominated and 25 patients were born or lived in goiter regions before puberty. Nine cases have died and had an average survival of four years and one month. Vascular disease was a primary or complicating factor in all the deaths. Sixty-four per cent had toxic adenoma. Evidence is presented which would indicate that, following thyroidectomy, the carbohydrate metabolism was improved. The operative mortality rate was 2.38 per cent. Preoperative and postoperative care is discussed.

Subclinical adreno-genital Syndrome. Glass, S. J., and H. C. Bergmann.

That the adreno-genital syndrome is usually associated with some degree of sex reversal is well established. Heretofore clinical differentiation of the benign and malignant forms of the disease was not possible without surgical exploration. X-ray visualization with perirenal air injection may demonstrate the gross tumour or hyperplasia of the adrenal. Quantitative sex hormone assays readily differentiate the malignant from the benign varieties by virtue of differences in the concentrations of the urinary androgens and estrogens; those with malignant tumors yield tremendous excesses of both sex hormones, whereas, the benign subclinical cases show relatively mild variations from normal sex hormone ratios. Thus such data makes possible the segregation of the surgical from the nonsurgical cases and will obviate many hazardous surgical explorations of the adrenals. This report of 12 male and female patients with benign adreno-genital dysfunction confirms the above sex hormone differences.

The effect of the administration of carbontetrachloride on the extent of regeneration in the enucleated adrenal of the rat. Higgins, G. M., and D. J. Ingle. To appear in this Journal.

This report covers our study of the effect of giving carbontetrachloride to rats upon the extent of regeneration of the left enucleated adrenal, when the right adrenal was removed. Eighty male rats from 180 to 189 gm. were used. The operation, involving the removal of the right adrenal gland and the enucleation of the left gland has been described. On the average, this operation provides a remnant of left adrenal, weighing approximately 2.54 mg. Animals upon which the effect of the drug was tested breathed the fumes for 30 to 40 minutes each day. Five animals were killed at 2 weeks, 5 at 3 weeks, 5 at 4 weeks, and 14 at 6 weeks following operation. The regenerated left adrenal was quickly removed and weighed. A group of animals, similarly operated upon constituted the control group. These were killed at comparable intervals, and their left adrenal weights were contrasted with those obtained from animals breathing the drug. The following conclusions were made: *a)*, Regeneration of the left adrenal occurred rapidly in the two groups. The average weights of the restored left adrenal in the carbontetrachloride-treated animals were slightly greater at 2 and at 3 weeks than the weights of their operated untreated controls. These differences, however, were probably owing to chance; *b)*, The effect of the drug on regeneration of cortical tissue was marked at 4 weeks after operation, and even more marked at 6 weeks after operation; *c)*, Carbontetrachloride, when administered for brief periods daily to rats, from which the right adrenal was removed and in which the left was enucleated stimulated greater amounts of cortical regeneration than

occurred in controls; *d)*, It is postulated that this increased impetus to regeneration of adrenal cortex is mediated through the pituitary gland.

The excretion and fate of androgens. II. Concerning the conversion of androgens to estrogens. Kochakian, C. D. To appear in this Journal.

The subcutaneous injection of the following androgens: urinary androgen extracts (a mixture of androsterone, dehydroandrosterone, isoandrosterone and epietiocholandiol) androstenedione, testosterone, testosterone benzoate, testosterone oxime, testosterone acetate and testosterone propionate into 1 normal and 2 castrate dogs did not result in the excretion of estrogens in the urine, as determined by the Allen-Doisy rat and mouse vaginal smear method. It seems that the male animal is unable to convert androgens to estrogens. The presence of this latter material in the male organism is probably due in part to an independent production by the testis, and the remainder to some extragonadal source.

Effect of previous castration on weight loss and survival of adrenalectomized rats. Kroc, R. L. To appear in this Journal.

Gonadectomy of 28 female rats, 6 to 20 weeks prior to 2-stage adrenalectomy, had no significant effect on the degree of weight loss, percentage of mortality, or length of survival. Six to 7-weeks castrated males lost an average of 3% more weight, and lived 3 days longer than normal males. The results on the castrated males are almost identical with those of normal and castrated females. Removal of the second adrenal when in estrus appears to have no effect on the survival period in rats.

The induction of premature puberty with androgenic substance. Kunstadter, R. H. To appear in this Journal.

Premature puberty was induced in two boys age 11½ years and 10⅔ years respectively, by the intramuscular injection of testosterone propionate. At the onset of treatment both boys presented small genitalia which were not considered dystrophic. Between 10½ and 11½ years, the first patient received several courses of intramuscular injections of phyone with some increase in growth of stature but no significant increase in size of the genitalia. Puberty developed rapidly during the administration of androgenic substance, at 11½ years of age. The second patient received a total of 2700 R.U. of APL at ten years for cryptorchidism. The testes descended, but during the following 8 months remained underdeveloped. His habitus was decidedly feminine and no secondary sex characteristics had developed. At the age of 10⅔ years androgenic substance was administered with subsequent rapid appearance of puberty. In view of the results obtained in these two patients, it is felt that androgenic substance should be administered with caution to children before the age at which puberty usually appears.

The inhibition of lactation during the puerperium by testosterone propionate. Kurzrok, R., and C. P. O'Connell. To appear in this Journal.

Twenty-one cases of early lactation during the puerperium were treated with testosterone propionate. The purpose of this was to inhibit lactation. Usually the inhibition of lactation by the generally used therapeutic measures, and especially in their absence, is associated with pain, tenderness, engorgement and lumpiness of the breasts. Such breast symptoms require considerable nursing care and are very distressing to the patient. Testosterone propionate, in doses of 25 mg. usually relieves such symptoms in about 24 hours without the addition of any other therapeutic measures. The hormone is injected twice a day, intramuscularly in the buttocks. A total dose of 100 mg. may be given within 48 hours, although if complete relief occurs after the 2nd or 3rd dose no additional doses need be given. The puerperium was not otherwise affected by the hormone and there were no unpleasant after effects.

Inadequacies of estradiol substitution in ovariectomized albino rats. Lauson, H., C. G. Heller and E. L. Sevringhaus. To appear in this Journal.

Injection of 0.25, 0.5, and 5.0 gamma of estradiol daily into 50 ovariectomized adult female rats for 20 days failed to suppress the gonadotropic potency of the hypophysis to anywhere near the normal level, as judged by the uterine and ovarian reactions in immature female test rats. A total of 153 donor rats were employed, and the pituitaries were assayed on 334 test animals. All doses of estradiol caused pituitary hypertrophy, whereas the usual thymus hypertrophy following castration was prevented by the two lower doses. In the high dose group, the thymus actually atrophied below normal level. It was concluded that daily injection of estrogen into castrate female rats fails to replace completely the normal ovarian

endocrine function, because doses too small to keep gonadotropic potency down to normal, or even to approach normal levels, already overstep physiological limits as regards pituitary and thymus weights.

The therapeutic effect of adrenal cortex extract on the psychotic patient. Loehner, C. A. To appear in this Journal.

Eleven patients suffering from mental diseases were used in this experiment. Nine of them were treated with a low potassium diet, sodium chloride, calcium lactate, and adrenal cortex extract (eschatin; Parke, Davis & Co.) by intramuscular injection, and 2 with eschatin alone. The following physiological changes were observed: the blood pressure dropped approximately 10 mm. of mercury in both systole and diastole, except in 3 cases of hypoadrenalism where it rose to the normal level; reversal of the mood to euphoria and elation; a feeling of warmth, drowsiness and relaxation was followed by normal, restful sleep without the use of sedatives; there were improved bowel movements and a disappearance of cyanosis of the extremities. One patient showed a remission of her mental symptoms, 2 were much improved, 6 were somewhat improved, whereas 2 were unimproved. The duration of the symptoms was from 6 months to 10 years. The average duration of mental symptoms was 3 years. One patient had severe pneumonitis, with very low blood pressure and marked leucopenia. He recovered from his acute infection and also showed a definite improvement in his mental condition. The treatment is safe, simple, and apparently there are no contra-indications to the injection of cortical extract. The patient is not subjected to dangerous, convulsant drugs, and there are no emergencies. No special training nor specialized nursing care is necessary.

Ocular transplants in female rabbits. Melchionna, R. H., and Sarah Flanders. To appear in this Journal.

Female rabbits with ocular homioplants of the seminal vesicle were studied daily for variations in the size and character of the transplants by direct observation and by a photographic method. At the termination of each experiment, histological studies of the transplanted tissues were made. Observations were recorded for spontaneous changes before and after castration and for responses to injections of androgen, estrogen, the gonadotropic substance of pregnancy urine and the corpus luteal hormone. In most instances, the results in the female rabbits were similar to those previously observed in male animals and may be summarized as follows. Vesicular transplants in the anterior chamber of the eye of female rabbits show the normal daily variations in size observed in similar transplants in male rabbits. The response of ocular transplants of the seminal vesicle in both male and female rabbits to injections of androgens and estrogens demonstrates that vesicular tissue is sensitive to both sex hormones, and is therefore a true ambisexual tissue. The increase in size of the transplants after injections of either androgen or estrogen is caused by hypertrophy and hyperplasia of smooth muscle, hyperplasia of epithelium and congestion of the blood vessels.

The specific dynamic action test in castrated and menopausal women. Moffat, M. To appear in this Journal.

An analysis is presented of 150 specific dynamic action tests performed on 100 patients, 50 at the Santa Barbara General Hospital and 50 at the Santa Barbara Clinic, the two laboratories having worked independently. The S.D.A. was determined 2 hours after a meal, which consisted of the whites of 3 hard-boiled eggs, a thin slice of toast and a cup of tea. Sixty-one tests were made in 34 women with active menopausal symptoms. Twelve of the women were castrates and 22 were in the natural menopause. The results are compared with those obtained in the other 66 patients who serve as controls. No significant difference between the two groups is found in either the average S.D.A. or the percentage of patients who showed a low S.D.A. The increased tendency toward obesity in the anovarian women is not a factor in lowering the S.D.A. Therefore the test is of doubtful value in the diagnosis of ovarian deficiency; and if menopausal symptoms are due to an over-activity of the anterior lobe of the pituitary, then this test is of doubtful value as a measure of anterior pituitary function as well.

The treatment of acne vulgaris with testosterone propionate. Molitch, M. To appear in this Journal.

Twelve young men with various types and degrees of acne vulgaris were treated with intramuscular injections of testosterone propionate over a period of 3 months. Ten similar patients were given injections of a bland oil and served as controls. The degree of improvement, both subjective and objective, was about equal in both groups. Seven additional patients

were treated locally with an ointment containing testosterone propionate with similar results. The high percentage of improvement obtained in both groups suggests that a marked psychogenic element was present in our cases. No increase in the incidence of nocturnal emissions or erections was noted.

Studies of the effects of pituitary extracts on carbohydrate and fat metabolism. Neufeld, A. H., and J. B. Collip. To appear in this Journal.

Results reported herein indicate that there is obtainable from pituitary tissue a fraction which is thermostable within a wide range of pH. This fraction has been demonstrated to have the following physiological properties: *a)*, Production of ketonemia in both normal and adrenalectomized rats; *b)*, Production of increased glycosuria and ketonuria in the Houssay dog; *c)*, An antagonistic action on the hypoglycaemic action of insulin and the hyperglycaemic action of adrenalin; *d)*, A glycostatic action which has been demonstrated in rats, guinea pigs and rabbits.

Photo-colorimetric method for the determination of androsterone in urine. Neustadt, R. To appear in this Journal.

A photo-colorimetric method is described for the quantitative determination of androsterone in human urine. The method is easily and quickly performed (in 5 to 6 hours), reduces outlay of work, practically excludes subjective error and yields an exact calculation of the secreted amount of androsterone in milligrams, international units or gamma per liter.

The specific metabolic principle of the pituitary, and its relation to the melanophore hormone. O'Donovan, D. K., and J. B. Collip. To appear in this Journal.

A specific metabolic stimulant present in pituitary extracts is described; it increases the oxygen consumption of rabbits, and depresses the respiratory quotient; CO_2 production is increased, especially in fasted animals and body temperature rises; there is evidence of decreased nitrogen metabolism. The hypothesis that fat metabolism is stimulated is advanced; but the depression of R.Q. being large relatively to the increase in oxygen consumption, it appears that there must be in addition some suppression of carbohydrate oxidation, or some increase of gluconeogenesis. The active principle is thermostable, resistant to alkali and to pepsin, destroyed by trypsin, and adsorbed upon charcoal. In these respects, and in its occurrence in extracts of various types, it exactly resembles the melanophore-expanding hormone. Reasons are given for distinguishing the active principle from the thyrotropic, adrenotropic, and growth hormones of the anterior lobe, and the pressor and oxytocic substances of the posterior lobe. Active extracts have been prepared from ox, sheep and pig pituitaries; the active principle is present in both anterior and posterior lobes but has been found in highest concentration in pituitary colloid and in extracts of the pars intermedia.

Pituitary cachexia? Osgood, E. E. To appear in this Journal.

A woman, 29 years of age with an eight-year history of amenorrhea, progressive loss of weight, and loss of teeth, was 5 feet, 3 inches (160.0 cm.) tall and weighed only 58.5 pounds (26.6 kgm.) The basal metabolic rate was low, as was the blood sugar curve. Her strength and appetite were good; yet on forced feeding she gained only about 15 pounds (6.8 kgm.) in a period of several years. Although she presented all of the clinical manifestations of pituitary cachexia, at necropsy the pituitary and other endocrine glands were essentially normal. No explanation of this syndrome was found.

A study of the estrogenic content of the tissues in pregnancy. Parker, F., Jr., and B. Tenney, Pr. To appear in this Journal.

Five cases of maternal and fetal organs have been extracted and assayed for their estrogenic substances and titrated against the placental content. In addition, the organs of 6 fetuses have been titrated against their placentas. The livers and adrenals of both mother and fetus have been studied because of their known involvement in the cholesterol metabolism. The effect of decomposition of the tissues on the amount of the estrogens extracted has been studied. The results have led us to believe that the source of the estrogens during pregnancy is in the general cholesterol metabolism. The placenta plays a prominent part but is not the actual source.

The effect of an anterior pituitary substance on the development of the human uterus. Payne, S. A., and E. K. Shelton. To appear in this Journal.

Ten patients with small undeveloped uteri ranging in ages from 19 to 32 years were

treated with a gonadotropic extract derived from pituitary substance. Menstruation was abnormal in all cases. Following treatment, irregularities of menstruation were favorably influenced in 7 and pregnancy occurred in 1. Endometrial biopsy specimens in those responding to treatment showed a tendency toward more normal histology including a secretory phase which had not previously been observed. Uterine growth was demonstrated in 3 instances; 2 were not improved by treatment.

Skeletal changes and blood calcium level in pigeons receiving estrogens. Pfeiffer, C. A., and W. U. Gardner. To appear in this Journal.

Injection of estrogen into the pigeon was followed by a rise in the serum calcium level and hypercalcification of the bone. The serum calcium of the normal male and the non-laying female pigeon, maintained under laboratory conditions, varied between 8.00 and 8.80 mg. per 100 cc. of serum. Following daily injections of 1,000 I.U. of estrogen these values increased, ranging from 13.12 to 21.65 mg. over a period of 10 weeks. Hypercalcification of the marrow-containing bones occurred normally at the time of ovulation and was correlated with the size of the ovarian follicles. Hypercalcification began in males after 2 weeks of treatment with 1,000 I.U. of estrogen daily. It reached a maximum after 5 weeks. There was a gradient of calcification beginning with the femur and extending to the tibia and then to the radius and ulna. Most of the new bone was endosteal although there were some periosteal deposits near the epiphyses. The osteoblasts were first active at the endosteal border. Then osseous spicules extended into the marrow and interlaced to form spongy bone which occupied most of the former marrow space.

Effect of long-term injections of testosterone upon the guinea-pig endometrium. Phelps, Doris, J. C. Burch and E. T. Ellison. To appear in this Journal.

Adult female castrated guinea pigs injected with 1 mg. of testosterone propionate daily for 34 days showed typical Swiss cheese dilatation of the endometrial glands and a mild generalized reaction of the endometrium. The glandular dilatation so produced was entirely comparable to the glandular dilatation produced by the injection of 2 R.U. of theelin daily for 30 to 40 days. The generalized reaction of the endometrium was similar in type to that produced by theelin but was much milder in degree.

The adrenal cortical syndrome in preadolescent girls. Reilly, W. A., H. Lisser and F. Hinman. To appear in this Journal.

The term 'pseudosexual precocity' is used to describe the syndrome produced in a little girl by a tumor of the adrenal cortex. It represents a sort of masculinization with early appearance of pubic hair and marked enlargement of the clitoris. Occasionally the breasts are developed prematurely. There may be more or less extensive hirsutism. Early menstruation is exceptional. This syndrome is to be differentiated from 'true sexual precocity' caused by a granulosa cell tumor of the ovary, which elaborates excessive amounts of the female sex hormone. Menstruation in the first 3 or 4 years of life is the rule with well-developed internal genitalia, and adult secondary sex characteristics. Enlargement of the clitoris does not occur. The literature appertaining to adrenal cortical tumors in little girls has been thoroughly reviewed. After careful scrutiny, 40 cases were considered proved instances of the syndrome. These tumors ordinarily are well encapsulated, permitting complete removal. This is fortunate, since often they become malignant. The surgical technic is discussed. A typical instance in a girl, 22 months old, is reported in detail, with illustrations. The tumor was successfully removed in May, 1935, with disappearance of the pubic hair and shrinking of the clitoris. The patient is well.

Comparative effects of light stimulation and administration of gonadotropic hormones on female sparrows. Riley, G. M., and E. Witschi. To appear in this Journal.

Female sparrows were exposed to lengthened daily light periods during fall, winter and spring. Exposure periods ranging from 21 to 75 days induce only a moderate degree of ovarial development. Female sparrows (including adults and juveniles) are partially refractory to injections of gonadotropic substances in September and October. Later in the winter fewer refractory cases are found, and none after February. Ovulation and eggshell formation were observed in several cases. The ineffectiveness of additional daily light periods in the production of an extensive ovarial development indicates a low degree of responsiveness of the female pituitary to light. It is suspected that behavioristic factors, ordinarily involved in the nesting and mating activities, and which were not given free play in our

experiments, are in some way very essential for the final hypophyseal stimulation and the growth of the ovaries to the breeding condition.

The effect on the growth rate of thyro-parathyroidectomy in newborn rats and the subsequent administration of thyroid, parathyroid and anterior hypophysis. Salmon, Theodora N. To appear in this Journal.

Thyro-parathyroidectomy of newborn rats resulted in severe retardation of growth. After 3 weeks the operated animals remained in a growth plateau at about 25 to 35 gm. They showed many of the symptoms of cretinism, including the susceptibility to infection, which limited the time they could be kept alive to about 2 months. The growth plateau was not related to weaning or to any demonstrable factor in mother rat's milk. Complete repair was not obtained when newborn thyro-parathyroidectomized rats were fed thyroid powder beginning at weaning time. Operated animals given parathyroid injections beginning with the time of operation showed a growth curve not significantly different from that of untreated operated animals. Normal growth response was not obtained when newborn thyro-parathyroidectomized rats were given thyroid alone beginning at the time of operation. Normal growth curves were obobtained in thyro-parathyroidectomized newborns when they were fed thyroid and given parathyroid injections. Pituitary implants administered to newborn thyroidectomized rats after weaning time did not produce a growth response if the animal was completely thyroidectomized. Only where thyroid remnants were demonstrable was a growth response noted.

The relation of environmental temperature to the action of thyroxine. Schmidt, L. H., and Ida Genther Schmidt. To appear in this Journal.

Preliminary experiments showed that lesions of the gastric mucosa occurred frequently in guinea pigs that had been exposed to a hot humid environment and had subsequently ingested small amounts of thyroxine. These experiments were designed to determine whether this increase in toxicity of thyroxine could be attributed to the high environmental temperatures. Male and female guinea pigs were exposed to a normal temperature (20°C.) and a high temperature (32°C.) for 2, 4, and 6 weeks, after which some of the animals were fed 0.6 to 1.0 mg. of thyroxine and then sacrificed. Gastric lesions were never found in any of the untreated or control groups at 20° nor in any of the untreated animals at 32°. Almost 50 per cent (43 of 48 animals) of the pigs developed ulcers following treatment at 32°. There was a noticeable difference in the reaction of the sexes to the high temperature and thyroxine treatment. Males kept at 32° for two weeks behaved like 20° males; ulcers developed frequently in females so treated. After 4 weeks the gastric lesions occurred in equal proportion in both sexes. After 6 weeks those females that survived treatment were resistant to thyroxine, whereas the males reacted as after 4 weeks. It has been suggested that the increase in toxicity of thyroxine at high environmental temperatures may be due to adrenal exhaustion.

The influence of various physiological substances on the glycogenolysis of surviving rat liver. I. methods; influence of the bile salts. Seckel, H. P. G. To appear in this Journal.

Glycogenolysis is studied by determining glycogen in surviving rat liver slices (pH 7.4, 37°C, 60 minutes, air atmosphere) without and with addition to the suspension solution of two conjugated bile salt preparations. At concentrations up to 0.2%, the bile salts inhibit glycogenolysis by 10 to 40% of the control figures. At 0.3 to 1.0%, they increase it by 10 to 50% or more. The significance of these findings for the pathology and treatment of Von Gierke's glycogen storage disease is discussed.

The influence of various physiological substances on the glycogenolysis of surviving rat liver. II. The influence of insulin added in vitro. Seckel, H. P. G. To appear in this Journal.

Under the same experimental conditions as described in the preceding communication, glycogenolysis of surviving rat liver slices is found to be inhibited by 10 to 43% by in vitro addition of insulin in concentrations of 0.3 to 6.4 U cc. of suspension solution (optimum at 4 U cc.).

Chick thyroid response as a basis for thyrotropic hormone assay. Smelser, G. K. To appear in this Journal.

The effect of injected thyrotropic extract on the thyroid weight of day-old-chicks has been studied and compared with other methods of testing for this hormone. The chick thyroid is an exceedingly sensitive test for this material, reacting to one-tenth the amount required to

affect the weight of guinea-pig thyroids. Chick thyroid weight increases with the amount of hormone injected over a wide range of dosages, whereas the guinea-pig thyroid weight increases over a narrow range. Chick thyroid response is consistent, significant values being obtainable with small groups. Administration of the total doses of thyrotropic extract in multiple injections greatly increases the response. Crude non-pituitary extracts, urine, and blood serum do not affect chick thyroid weight. Thyroid weight of normal guinea pigs is exceedingly variable, even when considered in terms of body weight, thus requiring that animals used for assay be within a narrow weight range. Histological response is elicited by amounts of extracts too small to produce hypertrophy and is, therefore, the more sensitive test, although not as adaptable to quantitative assay. If it is desired to demonstrate small amounts of thyrotropic principle, both histological structure and thyroid weight should be taken as criteria of an effect. A statistical treatment of assay data is advocated.

Effect of hyperthyroidism upon growth and chemical composition of bone. Smith, Evelyn E., and F. C. McLean. To appear in this Journal.

Thyroid was fed in Bills' normal stock diet in amounts sufficient to induce severe hyperthyroidism to young rats during the period of active growth and to older rats after decrease in active growth. The animals were killed between 4.5 and 8 months of age. Chemical analysis of the femurs showed no significant differences between the composition of the bones of the hyperthyroid and normal animals, with the exception that the water content and mean weight of the fresh femurs were significantly higher in the treated than in the control animals. There was no significant difference between the ash content of the bones of the control and experimental animals. Histological examination of the epiphyseal junctions of the long bones showed a cessation or marked retardation of growth in both young and old hyperthyroid rats. The cartilage was in a resting state, and was frequently perforated. Osteoblasts were few in number or absent. It is concluded that feeding of thyroid does not produce a specific catabolic effect upon calcium deposits in bones, but that it may lead to premature cessation or retardation of growth.

Influence of avitaminoses on weights of endocrine glands. Sure, B. To appear in this Journal.

The influence of various avitaminoses on the weights of the endocrine glands has been investigated. The most significant observations are as follows: In vitamin-A deficiency, there was observed an hypertrophy of the pancreas amounting to 40%. In vitamin-B_1 deficiency there is a marked hypertrophy of the thyroids and adrenals. The most noteworthy change in endocrine weights in avitaminoses is the marked atrophy of the thymus in vitamin-B_1 deficiency which occurred in 100% of the pathological animals. The atrophy was correlated with histological observations. Hypertrophy of the pituitary was found in vitamin B_1 and B_2 deficiency to the extent of 40 and 25% respectively. Repeated vitamin-B_1 depletions on endocrine weights have the following effect: The hypertrophy of pituitary entirely disappears; in the thyroid it is reduced to insignificant proportions (9.1 %) and in the adrenals it is reduced from 50 to 20%.

The oral administration of hormonal proteins: thyroid protein and insulin. Wilson, Hildegarde, T. S. Sappington and W. T. Salter. To appear in this Journal.

The 'inactivation' of insulin by proteolytic enzymes in vitro may be reversed in whole or in part. This finding suggests that the oral administration of insulin may be effective under appropriate circumstances. In mice, in fact, the oral administration of insulin preparations has consistently and reproducibly produced hypoglycemic coma or convulsions within 2 hours after administration. The oral dose observed for undigested insulin hydrochloride was about 90 times its subcutaneous dose. The oral dose observed for digested insulin was about 45 times its subcutaneous dose. Iron salts increased the effectiveness of insulin and digests of insulin when administered orally. These observations suggest qualitative similarities between thyroglobulin and insulin with respect to the action of proteolytic enzymes. They suggest, further, that hormonal proteins can be used orally under suitable conditions.

ADRENALS

Intersexuality and suprarenal virilism. (Intersex mit suprarenalem Virilismus [Knochenmarkentwicklung in den hyperplastichen Nebennieren]) Bosselmann, H., Endokrinologie 19:292. 1937.

A case is described with autopsy report of a 58-year-old person with ovaries, tubes, uterus,

prostate, seminal vesicle, hypertrophied clitoris, male type of hair distribution, moderate hypertrichosis on the trunk and the extremities, hypersecretion of the sebaceous glands dating from the 16th year, and hypertrophy of the adrenal cortex, showing the possibility of the coincidence of intersexuality and suprarenal virilism. Attention is also called to the presence of symmetrical masses of bone-marrow tissue in both suprarenals in an amount greater than any previously described.—W. J. A.

Chemical studies on the adreno-genital syndrome I. The isolation of 3(α)-hydroxyetiocholane-17-one, 3 (β)-hydroxyetioallocholane-17-one (isoandrosterone), and a new triol from the urine of a woman with an adrenal tumor. Bulter, G. C., and G. F. Marrion, J. Biol. Chem. **124:**237. 1938.

A preliminary examination was made of the ether-soluble neutral fraction of 45 liters of concentrated but unhydrolyzed urine obtained from a woman with an adrenal tumor and marked symptoms of virilism. The following steroids were isolated and definitely characterized: 3(α)-hydroxetiocholane-17-one, B(β)-hydroxyetioallocholane-17-one (isoandrosterone), and pregnane-3(α)17.20, triol. A new triol of the formula $C_{21}H_{36}O_3$ has also been isolated. It was precipitable by digitonin and on oxidation with lead tetracetate yielded a volatile aldehyde or ketone and a substance melting at 144-146°. The new triol was therefore probably a pregnane or allopregnane-3(β), 17.20-triol. Since the non-volatile lead tetracetate product is probably not isoandrosterone, the former possibility was preferred.—J. M. L.

An initial depression of heart rate in response to epinephrine in human subjects. Fuchs, R. T., J. Pharmacol. & Exper. Therap. **63:**143. 1938.

Epinephrine, 0.025 mg., was injected intravenously into 20 normal and 20 schizophrenic individuals. Records of systolic blood pressure showed that the average rise in blood pressure was about 30 mm. in both groups. The blood pressure returned to its initial value 4 minutes after injection. The heart rates decreased about 15 beats per minute after a latent period of 12 seconds, which was followed by an abrupt rise to about 30 beats above normal about 20 seconds later. Atropine was administered to 10 members of the schizophrenic group and the epinephrine experiments repeated. The average value of the depression in heart rate was reduced 72%. The period of accelerated heart rate was prolonged and the pressor effect was greater after atropine. The author believes the initial depression in heart rate represents a compensatory reaction of the parasympathetics to sympathetic stimulation.—E. L.

Is there a paralytic secretion from the medulla of a permanently denervated adrenal (Existe-t-il une sécrétion paralytique de la glande medullo-surrenale "chroniquement enervee")? Hermann, H., F. Jourdan, G. Morin and J. Vial, Compt. rend. Soc. de biol. **126:**13. 1937.

The adrenal vein of a test dog, whose adrenal glands had been denervated 40 days previously, was attached to the jugular vein of another dog whose lower spinal cord had been removed. The latter is very sensitive to small changes in the amount of circulating adrenin. In 7 of 9 test animals there was evidence of adrenin in the blood.—J. C. D.

Functional homeoplastic grafts of the adrenal gland of newborn rats. Higgins, G. M., and D. J. Ingle, Anat. Rec. **70:**145. 1938.

Adrenal glands of newborn rats were grafted to unrelated adrenalectomized adult rats. Forty adult rats were grafted: and of these, 24, or 60% were successful. Past experience has shown that homeografts of adrenal glands of adult rats rarely survive. Results of this study indicate that glands of newborn rats are more likely to grow and function. Grafted glands degenerate except for a small narrow zone of glomerulosa and the capsule. From these structures regeneration of functional cortical masses of tissue took origin. Medulla did not regenerate. Grafts which did not regenerate were infiltrated by lymphocytes, neutrophils and giant cells. Grafts will not survive or regenerate in the presence of functional adult adrenal glands. —Author's Abst. Wistar Bib. Service.

Three cases of chromaffin cell tumors of the suprarenal gland. Holst, E. J., Acta med. Scandinav. **94:**510. 1938.

Three cases of suprarenal gland tumor were studied. The chief symptoms were attacks of discomfort with pallor, cold and clammy extremities, palpitation of the heart, sensation of pulsation in the head and neck, headache, dizziness, nausea, vomiting, tremors, stenocardia, epigastric distress, midriasis, salivation and epiphora. There were frequent paroxysms of in-

creased systolic pressure with but slight changes in diastolic pressure. Two of the cases died after operation and the third of cerebral hemorrhage before operation. All 3 patients were between the ages of 17 and 25.—J. M. L.

Weights of adrenal glands in rats fed different amounts of sodium and potassium. Ingle, D. J., and E. C. Kendall, Am. J. Physiol. **122**:585. 1938.

Male rats were unilaterally adrenalectomized. To the basic diet 1% *K* was added for 1 group, for another 0.6% Na, and to the third no additional Na or *K* was added, and to the 4th 0.6% *K* and 1% Na were added. No variations in the weight of the adrenals of the different groups was noted within 6 weeks. This was in contrast to the results when animals were exposed to other types of stress (i.e., thyroxin or work).—Lena Lewis.

Modification of the vascular effects of adrenin by sex hormones of the opposite sex (Modifications de l'effet vasculaire de l'adrénaline par les hormones sexuelles de l'autre sexe). Karasek, F., and O. Poupa, Compt. rend. Soc. de biol. **126**:118. 1937.

In frogs sex hormones of the same sex reinforce the action of adrenin in producing a constriction of an artery. Those of the opposite sex reduce this effect materially. The results of 2 typical experiments are given.—J. C. D.

Chemical studies of the suprarenal cortex V. Conversion of compound E to the series which contains four atoms of oxygen and to adrenosterone by the action of calcium hydroxide. Mason, H. L., J. Biol. Chem. **124**:475. 1938.

A member of the $C_{21}O_5$ series, compound E, isolated from the suprarenal gland, was converted into one of the $C_{21}O_4$ series through rearrangement of the dihydroxyaceton group by treatment with saturated salcium hydroxide in an atmosphere of nitrogen for 48 hours. Adrenosterone was also obtained by this procedure.—J. M. L.

Chemical studies of the suprarenal cortex IV. Structures of compounds C, D, E, F, and G. Mason, H. L., W. H. Hoehn and E. C. Kendall, J. Biol. Chem. **124**:459. 1938.

A technical description of the methods used for the identification of compounds of the $C_{21}O_5$ series C, D, E, F, and G isolated from the suprarenal gland. Compound E was given a new formula identical with compound A with the addition of a hydroxyl group at C_{17}. Compounds C, D, F, and G differed from compound E only in the functions of the oxygen-atoms and in the presence or absence of the 4.5-ethylene bond. These formulae were in agreement with those of Reichstein. The carbonyl group at C_{11} of compound E and its degradation products does not show the usual ketone reactions. It does not add methyl magnesium iodide but behaves like highly hindered ketones which enolize and liberate methane. The hydrogenation of the $C_{20}O_5$ acid from compound E was studied.—J. M. L.

Influence of gestation-lactation and some pathological conditions on the weight of the suprarenal glands in the albino rat. Mutow, T., Tohoku J. Exper. Med. **30**:448. 1937.

The author experimented on 140 albino rats to determine the weight of their suprarenals under various conditions. Among them 44 were pregnant or suckling, 66 were free from signs of disease and 79 were diseased. The weight of the suprarenals was not influenced by infections, whereas it was greater in the pregnant-lactating rat than in the nonpregnant-lactating ones and greater at estrus than at diestrus.—Margaret Baptist.

Epinephrine output rate in dogs after sodium nitrite injection. Satake, Y., H. Sato and K. Abe, Tohoku J. Exper. Med. **31**:416. 1937.

Four dogs, which were prepared for the collection of blood from the suprarenal capsule, were given sodium nitrite intravenously in doses of 20, 30 and 40 mg. per kilo of body weight. Epinephrine was measured by the rabbit intestine segment method. Its output was accelerated definitely in all 4 cases from 3 to 10 times the initial rate.—Margaret Baptist.

Influence of application of cold to the dog's body upon the blood pressure and the blood sugar, with special reference to the augmented epinephrine secretion. Satow, Y., Tohoku J. Exper. Med. **30**:561. 1937.

Experiments were made on dogs to ascertain how the epinephrine secretion, augmented by a cold bath, affects the circulatory system and the carbohydrate metabolism. The body temperature continued to fall after removal from the bath with subsequent gradual recovery. The blood pressure and pulse rate rose at first, but later fell with the temperature. The blood-

sugar content increased rather slowly, but it fell to normal again before the temperature was restored. The removal of the suprarenal medulla exaggerated the body temperature fall, the blood pressure fall and the bradycardia, and prolonged the hyperglycemic period. Introduction of adrenalin hydrochloride in saline abolished these variations due to the loss of the suprarenal medulla. Thus, the augmented secretion of epinephrine from the suprarenal glands on cooling the dog excessively moderated the fall of the body temperature, the arterial blood pressure and the retardation of the heart beat, and cut short the hyperglycemic period.—Margaret Baptist.

ENDOCRINE GENERAL

Investigation of the diabetogenous hormone in urine. Bjering, B., Acta med. Scandinav. **94**:483. 1938.

Urine preserved with alcohol and chloroform was acidified with acetic acid and shaken with absorbent. After standing over night the precipitate was removed and eluted with 1% NH_4 OH. The active principle was precipitated with acetone and redissolved in water. The material derived from urines of healthy subjects, diabetic patients, and pregnant women was injected subcutaneously into rabbits, and the blood sugar determined every half-hour afterwards for 3 to 6 hours. It was found that normal urine contained small amounts of a blood-sugar raising principle, and there were relatively larger amounts in the urine of some diabetics and pregnant women. The use of insulin decreased the amount of substance excreted.—J. M. L.

The distribution of iron in blood. Jenkins, C. E., and M. L. Thomson, Brit. J. Exper. Path. **18**:175. 1937.

The authors described a colorimetric method of determining the distribution of iron in blood. They showed that the total Fe in blood is greater than can be accounted for by any known method of estimating the Hb concentration and thus confirmed the observations of Barkan and of Winegarden and Borsook. The average non-Hb cellular Fe is normally about 7%; the plasma Fe in males is about 0.3 mg. per 100 cc. of whole blood, and about 0.8 mg. in young women. In the latter it is at its highest concentration before and during menstruation and falls to its lowest level immediately after menstruation has ceased. They suggested that this indicates that the rate of regeneration of blood in the young female is in part influenced by the menstrual cycle. In the anemias they found that the non-Hb cellular Fe and the plasma Fe differ largely from the normal. It was concluded that the estimation of whole blood Fe as a means for calculating the Hb concentration is valueless.—Margaret Baptist.

An endocrine cardiac syndrome (Le syndrome endocrino-cardiaque). Oumansky, V., and A. Longuet, Presse méd. **46**:388. 1938.

Symptoms of cardiac insufficiency are sometimes associated with melanodermia and cirrhosis. The patients have a history of endocrine disturbances, mostly hypogonadism. A case reported was completely relieved by treatment with adrenal cortex extract.—A. E. M.

Nervous and hormonal regulation of basal metabolism (Die nervös-hormonale Regulation des Grundumsatzes). Winkler, W., Wien. Arch. f. inn. Med. **32**:241. 1938.

The author gives a critical review based on his own observations and those of several other investigators. In his summary he states that the total of the basal metabolism is the sum of the resting metabolism of the tissues, especially the musculature and the liver. Three internally-secreting glands, the pituitary body, the thyroid and the adrenals, influence it, in part directly and in part through the peripheral and central autonomic nervous system. This action is seldom individual but mostly by various interactions. The harmonizing and balancing of these several factors is brought about by a basal metabolism-regulating center located in the midbrain.—W. J. A.

GONADS

Hyperplasia and hypertrophy of the uterine musculature in ovariectomized rats following estrone injections. Barks, O. L., and M. D. Overholser, Anat. Rec. **70**:401. 1938.

Daily subcutaneous injections of estrone (theelin-in-oil) were made in rats ranging in age from 80 to 156 days, after castration periods of 1, 2 and 3 months. Four dosages of estrone were used: *a)*, 5 R.U. for 15 days; *b)*, 10 R.U. for 10 days; *c)*, 15 R.U. for 10 days; *d)*, 25 R.U. for 30 days. Estrone caused a definite increase in the length and diameter of the horns and bodies of the uteri. This increase in size was due in part to an increase in the thickness of the musculature. The total muscle thickness in the injected castrated animals was 0.4308

mm., while in the castrated control animals it was 0.1969 mm. The circular layer underwent a greater increase than the longitudinal layer. A study of the sections showed that hypertrophy of the muscle cells had occurred. Transverse sections of the uterine horns were divided into quarters by ink lines marked on the cover slips. The total number of longitudinal muscle fibers in one-quarter of the section was counted. The same region was compared in the experimental animals and controls. Counts in injected animals ranged from 1232 to 3932, with an average of 2121 fibers, while in the castrated controls the counts ranged from 500 to 1707, with an average of 1040 fibers. These figures definitely show that hyperplasia of the muscle fibers also occurred. Variations in the estrone dosage and time of castration atrophy did not result in any significant differences.—Author's Abst., Wistar Bib. Service.

About the hormonal treatment of cryptorchism. Sand, K., Endokrinologie **19**:306. 1937.

A preliminary report is made of the case of a boy of 10 years old having bilateral abdominal testes, a slight degree of dystrophia adiposogenitalis and moderate mental retardation. During a rather intensive hormonal treatment with preparations of prolan (Antex Leo, Physex Leo, and Mecosex) and andrin (Diorchistin, Neoorchistin and Testex) during ¾ of a year the 2 testes have descended into the scrotum where they have remained and grown. The boy has shown simultaneous favorable physical and mental development. The author maintains a conservative attitude as to how much importance may be attributed to this special 'combined' method of treatment.—W. J. A.

Activity of follicle hormone in initiating labor in women (Ueber die Wehenauslösende Wirkung des Follikelhormones bei der Frau). Streit, K., Zentrlbl. f. Gynäk. **62**:1252. 1938.

This is a report of 23 women who had delayed onset of labor at term, or in whom labor pains were weak, infrequent, or subsided without progress. They were treated with estrone acetate intravenously, total doses of 100,000 to 6,000,000 I.U., in 1 to 10 injections. Doses of 1,000,000 U were well borne. In 15 cases there seemed to be initiation of uterine activity soon enough to attribute it to estrone. In some cases quinine or posterior pituitary preparations were added later, with effective improvement. It seems that the use of estrone is more helpful in initiating labor than in increasing the activity of a hypoactive uterus.—E. L. S.

Duration of secretory activity in the glands of the decidua vera. Sturgis, S. H., Am. J. Obst. & Gynec. **35**:752. 1938.

When pregnancy is complicated by an ovarian tumor operative removal of the tumor may involve the corpus luteum. Evidence from the literature suggests that excision of the corpus luteum results in from 10 to 33% of miscarriages within the first 2 months. This evidence, however, is neither statistically accurate nor reliable. A satisfactory test for the presence of the luteal hormone would lead to a better understanding of the risks involved in removing the corpus luteum in early gestation. The characteristic response of decidual glands to progestin is the only available test, at present, for this hormone. The duration of this response was studied in surgical specimens of 13 uteri removed at approximately 3-week intervals through pregnancy. Photographs of some of these sections are reproduced to demonstrate progressive changes in these glands. The progressive degeneration of decidual glands demonstrated in this study suggests that their secretory function is limited by an intrinsic exhaustion rather than a decrease in the stimulating mechanism. The maximum involvement of decidual glands in active secretion is found at about the 8th week in the superficial zones. This would appear to lend a certain amount of support to conclusions from evidence in the literature that the corpus luteum cannot be excised within 8 weeks from the last menses without seriously jeopardizing the successful course of gestation.—Author's Abst.

Anterior pituitary gonadotropic and anterior pituitary-like hormones in the treatment of cryptorchidism. Turner, H. H., South. M. J. **31**:38. 1938.

Analysis of the literature showed 320 treated cases; of these 60% showed complete descent, 9% partial descent and the remainder no significant improvement. Turner treated 26 cryptorchid patients with anterior pituitary-like hormone. Descent of the testes has been obtained in 14 (53.9%), partial success in 8 (30.8%), and failure of descent in 4 (15.3%). The average length of time of treatment was 25 weeks, with a total dosage of 13,000 R.U., corresponding to about 500 U weekly. In general, no serious complications have been observed, although in one patient precocious sexual development and, in another, softening of the testicle have been suggested. Endocrine therapy is of definite value as a preliminary to and following

orchidopexy. The increased size of the testicle and lengthening of the cord elements permit better surgery, and the postoperative results are greatly improved by the treatment.—R. G. H.

HYPOPHYSIS

The effect of administering frog anterior pituitary substance to immature female mice. Adams, A. Elizabeth, and Gertrude Tukey, Anat. Rec. 71:1. 1938.

Injections of saline suspensions of 16-96 fresh or acetone-dried anterior pituitaries of frogs (Rana pipiens) were given daily for 4 days to twenty-five 18- to 19-day-old, and six 21- to 22-day-old female mice and the hosts killed 96 to 100 hours after the first treatment. Litter-mate mice received equal amounts of anuran brain tissue. One litter was given mouse anterior pituitary or brain for comparison. No precocious sexual maturity, i.e., maturing of ovaries and of the reproductive tract, was induced in the mice by injections of frog pituitary, but homoplastic pituitary administration gave a positive reaction. Hypertrophy and hyperplasia of the thyroid glands of the mice and hypertrophy of their adrenal cortices followed the treatment with frog pituitary substance. The adrenal hypertrophy was greater in those mice receiving 64 or more pituitaries than in those given fewer. Sixteen fresh glands called forth no reaction in the adrenals.—Author's Abst., Wister Bib. Service.

The anterior pituitary gland and carbohydrate metabolism. Anderson, Charlotte M., M. J. Australia 1:2. 1938.

Anterior pituitary extracts inhibited the normal mechanism by which glycogen is deposited in the liver of young rabbits, either following the administration of insulin or of sodium lactate. The pituitary extract did not appreciably increase the effect of adrenalin. It is concluded that the extract interrupts the Cori cycle of glycogen metabolism at the some stage.—G. B.

The biological assay of the carbohydrate metabolism hormone of the anterior pituitary. Bergman, A. J., and Turner, C. W., J. Biol. Chem. 123:471. 1938.

Intraperitoneal injections into guinea pigs were made of the material obtained by precipitation between 60 and 86% alcohol, at a pH of 5.7, of the acetone dried powder of anterior pituitaries of sheep. The animals were sacrificed 8 hours later and the blood sugar determined by the method of Shaffer and Somogyi. A unit was taken as the minimum amount of material which caused a 50% increase in the blood sugar of at least 5 animals. It was found essential to use animals that weighed between 180 and 220 gm. and were gaining normally in weight. About 250 U/gm. of dried extract was obtained both from sheep and cattle pituitaries.—J. M. L.

A new method for posterior pituitary assay. Coon, J. M., J. Pharmacol. & Exper. Therap. 63:5. 1938.

A satisfactory quantitative estimation of small quantities of the oxytocic activity of pituitary solutions can be obtained by measuring the rapid and transitory fall in blood pressure produced in a chicken anesthetized with phenobarbital.—E. L.

The effect of antithyrotropic serum on the action of the human thyrotropic hormone. Cope, C. L., Lancet 234:888. 1938.

Fifty human pituitary glands were collected from routine post-mortems over a period of 6 months and were desiccated with acetone. The active principle was prepared according to the method of Rowland and Parkes. The yield was 700 mg., having only 30% of the activity of a similar preparation obtained from ox pituitaries; 12 mg. represented the equivalent of 1 Parkes unit. Antithyrotropic serum was prepared in a pair of rabbits by the daily injection of 25 mg. of ox pituitary extract rich in thyrotropic activity. The injections were continued for 4 months. During the last 6 weeks the serum was strongly antithyrotropic, and was removed at intervals by bleeding from an ear vein. Four to 6 guinea-pigs received increasing doses of ox thyrotropic hormone subcutaneously for 5 days, and were killed on the 6th day. Although the antithyrotropic serum prepared in rabbits against ox thyrotropic hormone will antagonize action of this hormone, it had only a negligible inhibitory effect on the thyrotropic hormone obtained from the human pituitary. Therefore the author is doubtful whether such serum will prove of therapeutic value in the human subject.—L. B. S

The effect of eclamptic blood upon the urinary output and blood pressure of human recipients. Page, E. W., J. Clin. Investigation 17:207. 1038.

Amounts of pituitrin as low as 0.01 U were found to interrupt the diuretic curves of several suitably prepared human recipients. In 9 cases, the rapid transfusion of 500 cc. of normal blood did not result in a significantly altered blood pressure or urinary output, although the results suggest the presence of traces of postpituitary hormone in normal blood. If small amounts of pituitrin (0.1-6.0 U) were added to normal blood and allowed to stand in vitro for 30 to 60 min. before transfusion, definite antidiuretic responses were obtained in 6 experiments. When large amounts of pituitrin were given to normal donors just prior to the withdrawal of blood, no definite antidiuretic responses were obtained in the recipients, indicating a rapid elimination or destruction of the hormone from the circulating blood stream. Four hundred cc. of blood were rapidly transferred from 5 women with eclampsia or severe preeclampsia to normal (anemic) pregnant women, suitably hydrated, but there were no rises of blood pressure, interference with diuresis, or untoward symptoms. The results would not support the contention that there is a markedly toxic substance in toxemic blood, nor the theory that there is a hypersecretion of the postpituiary gland in eclampsia.—Author's Abst.

Surgical treatment of pituitary basophilism. Pattison, A. R. D., and W. G. A. Swan, Lancet **234**:1265. 1938.

Two cases of Cushing's syndrome in young women, aged 16 and 26, are presented, in whom improvement followed the insertion of radon seeds into the sella turcica. Both cases had proved resistant to deep radiotherapy.—L. B. S.

Factors determining voluntary ingestion of water in normals and in individuals with maximum diabetes insipidus. Richter, C. P., Am. J. Physiol. **122**:668. 1938.

The total water intake was carefully measured (i.e. ingested water, plus water content of the food, plus water of oxidation). Rats with maximum diabetes insipidus were used in these experiments, 530 with chronic symptoms, 240 with acute symptoms. Records of normal maximum intake of cats, monkeys, dogs and man were obtained (largely data of other workers). The average water intake of normal subjects is a function of the body area, rather than weight, varying from 1,050-1,238 cc./sq. meter body surface. It is therefore dependent on body metabolic rate. On the other hand, voluntary water intake in maximum diabetes insipidus is a function of body weight rather than body surface. That a maximum output had been obtained in rats was shown by the fact that no additional output occurred when water was forced. The daily intake of water for rats and humans was about 1,000 cc. per kg. and slightly lower in cats, dogs and monkeys. The ratio of the intake of the animal with diabetes insipidus to that of the normal was approximately the same for each species, whether based on body weight or surface area.—Lena Lewis.

The morphological basis of pituitary function in pigeons. Schooley, J. P., and O. Riddle, Am. J. Anat. **62**:313. 1938.

Cellular structure of the anterior pituitary was studied by uniform technical methods in doves and pigeons at known stages of growth and reproduction. Some of these stages are here emphatic and distinct in their indications of concurrent production or non-production of certain pituitary hormones. Such stages therefore provide unusual bases for determining both cellular structure associated with active hormone production and the cell type associated with the production of specific hormones. Two types of chromophilic and one type of chromophobic cells are recognized. The evidence presented indicates that the chromophilic cells arise from chromophobes, that they acquire the cell organs of either basophils or acidophils and that these latter function as such during their life span. Distinct secretory phases are recognized in acidophils and basophils. Their diagnostic characters are described and correlated with events of the reproductive cycle. Anterior lobe tissue from pigeons, cows and rats in certain reproductive states were implanted into immature male ring doves to test their hormonal content. The results of these tests are reported and correlated with the cytological manifestations. The current conception that gonadotropic hormone arises from basophils is supported. Evidence is presented to show that prolactin arises from acidophils. Certain aspects of hormone production, of hormone storage and of a reciprocal control in pituitary and receptor glands are discussed.—Author's Abst. Wister Bib. Service.

The effect on the posterior pituitary principles of incubating beef heads at 37°C. for 20-24 hours. Vaichulis, J. A., J. Pharmacol. & Exper. Therap. **63**:37. 1938.

Pituitaries, dissected from beef heads incubated for 20 to 24 hours at 37°C., assayed 95-100% of the standard powder in both oxytocic and pressor activity.—E. L.

Test for prolactin based on smears from the crop gland of pigeons (Test de la prolactine basé sur le frottis de la muqueuse du jabot). Valle, J. R., Compt. rend. Soc. de biol. **126**:134. 1937.

A fistula was made into the lateral pouch of the crop gland in 6 adult pigeons and 5 squabs. The latter were artificially fed. When these birds were injected with a substance containing prolactin, smears taken from the pouch through the fistula showed changes within 2 or 3 days. The granules staining with Sudan III in the cells increased both in number and in intensity of staining.—J. C. D.

Arterial hypertension and vasopressive and gonadotropic hypophyseal hormones (Hypertension artérielle et hormones hypophysaires vasopressives et gonadotropes). Van Boggaert, A., and Fr. van Basile, Acta med. Scandinav. **96**:56. 1938.

An examination was made of the cerebro-spinal fluid from 19 patients with hypertension. Anterior pituitary gonadotropic hormones were estimated by injecting the detoxicated and concentrated spinal fluid into 30-gm. rats, and noting the appearance of the vagina and ovaries. The vasopressive posterior pituitary hormone was determined by its effect on the blood pressure when injected into the posterior cisterna of the dog. No evidence of a hypertensive hormone was found. In only 3 instances was a gonadotropic effect produced and in these cases clinical evidences of hypogonadism were present.—J. M. L.

Phypophysis and water metabolism (Hyperphyse und Wasserhaushalt). Wermer, P., Wien. Arch. f. inn. Med. **32**:189. 1938.

By experiments on dogs, and by observation of clinical cases as well as from a review of the recent literature, the author is led to stress the importance of the anterior pituitary in diabetes insipidus. It is found that from the anterior pituitary there may be extracted a substance with a marked diuretic action. This is contained in the same fraction as the growth hormone. Two varieties of diabetes insipidus are recognized: one primary in the anterior lobe and one primary in the posterior lobe. Both are due to lack of balance between the diuretic hormone of the anterior lobe and the antidiuretic hormone of the posterior lobe.—W. J. A.

Observations on hypopituitarism. A contribution to early diagnosis. Zondek, H., Acta med. Scandinav. **95**:437. 1938.

In 8 women with signs of hypopituitarism there were 5 with enlarged sellas and 3 with small ones. The chief symptoms in all cases were a tendency to obesity, menstrual irregularity, amenorrhoea and loss of libido, mental upsets usually with depression headache and generalized muscular weakness. The B.M.R. was usually within normal limits. The enlargement of the sellas was thought to be due to chromophobe adenoma. There is often chronic increased intracranial pressure, which can be relieved by injections of hypertonic glucose solutions and restriction on salt intake.—J. M. L.

PANCREAS

The biochemical mechanism of insulin and metrazol shock (Contribución al mechanismo bioquímico del shoc insulínico y cardiazólico). Cuatrecasas, J., and A. A. Bruno, Rev. sud-am. de endocrinol. **21**:224. 1938.

Insulin and metrazol have opposite actions on the carbohydrates in the organism. The effect on blood sugar of metrazol is not connected with the neurohumoral phenomena of the shock. The curative action of shock in schizophrenia is not related to the changes in sugar metabolism. In both types of shock there occurs an elevation of the plasma chlorides: blood corpuscles ratio.—A. E. M.

End results in uncontrolled mild diabetes. Dillon, E. S., and Dyer, W. W., Pennsylvania M.J. **41**:589. 1938.

Early diagnosis of diabetes mellitus must be made if disastrous end results are to be avoided. Education of the public and periodic health examinations should do much to bring the patient to the physician at an earlier date. Adequate control of the diabetic from the time of diagnosis must be secured. This means: *a)*, a balanced diet sufficient to maintain weight and strength, *b)*, a normal fasting blood sugar, *c)*, a 24-hour urine sample that was free from sugar, *d)*, the absence of hypoglycemic reactions. In the group of 100 cases forming the basis of this report, 66 were discharged from the hospital. With the exception of one patient who left against advice, the patients were considered adequately controlled diabetically. Proper

instruction must be afforded the patient. He must be taught the importance of following the prescribed dietetic and insulin regime. He must be taught the essentials of foot hygiene and the dangers resulting from failure to adhere to it. No patient is more conscientious about diabetes than one who has lost a leg. However, much of this personal and economic waste can be eliminated by proper instruction of the patients.—I. B.

Influence of cutting the vagi on the blood sugar curve after glucose or insulin (Action de la vagotomie sur les courbes des glycémies produites par le glucose ou l'insuline). Etcheverry, A. O., Compt. rend. Soc. de biol. **126**:147. 1937.

The vagi were cut above the diaphragm in 12 dogs. When they had recovered and were in good health, the blood-sugar curve was followed, after the injections of glucose and of insulin. Although the normal blood-sugar level was not modified, the response to either substance injected was much slower than in normal dogs. The vagus, therefore, shares in the control of the blood sugar and makes the responses to change more rapid and accurate than when the hormonal control acts alone.—J. C. D.

Protamine zinc insulin and insulin resistance (Protamin-Zinc-Insulin und Insulinresistenz). Falta, W., Wien. Arch. f. inn. Med. **32**:97. 1938.

From 150 cases 11 are chosen for detailed description. It was found that cases of insulin sensitivity as well as those of insulin resistance often show an improved tolerance when protamin zinc insulin is employed. The author holds that protamin zinc insulin has little value in early treatment of diabetic coma. He believes that protamin zinc insulin is of greatest value in cases of recent diabetes. He does not agree with Taubenhaus that old, insulin-resistant cases with nephrosclerosis show the best results with protamin zinc insulin.—W. J. A.

The significance of dermatophytosis in diabetes. Kelly, H. T., Pennsylvania M. J. **41**:581. 1938.

A serious problem always confronting the diabetic and his physician is gangrene, which next to coma is the most important preventable cause of diabetic death. Inasmuch as the Epidermophyton fungus primarily invades the tissues, thereby opening an avenue for secondary infection and consequent gangrene, it is essential to have a thorough understanding of its high incidence, recurrences, sources, repeated possibilities of exposure, characteristic clinical lesions, histopathology, and associated allergic manifestations to prevent gangrene in diabetics. Fonseca's vaccine has been of material aid in successfully managing 30 cases of dermatophytosis in diabetics without the occurrence of a diabetic gangrenous lesion.—I. B.

Syphilitic diabetes. With additional case reports. Kitchell, J. R., Pennsylvania M. J. **41**:587. 1938.

This is a brief review of the subject with 4 illustrative case reports. The recovery of patients with syphilitic diabetes after antisyphilitic treatment is more apparent than real, and eventual relapse into the diabetic state is probably the fate of all of them. It is believed that the patients reported who appear to be cured but who were followed only a short time and were not located for a check-up examination, have either relapsed or will relapse into diabetes. In the course of years, in spite of adequate and long-continued antisyphilitic therapy, these people usually become diabetic again, and after this relapse the disease must be treated like any ordinary case of diabetes mellitus.—I. B.

Nesidioblastoma, the islet tumor of the pancreas. Laidlaw, G. F., Am. J. Path. **14**:125. 1938.

This is a microscopic study of 9 adenomas of the islets of Langerhans (nesidioblastomata) removed surgically from 6 patients. The operations were performed for the relief of hypoglycemia with severe and long-continued collateral symptoms of from 1 to 12 years' duration. In 5 patients the blood sugar rose and the other symptoms disappeared promptly; the 6th did not recover until after a second operation, at which another tumor was discovered and removed. Microscopically the chief feature of most of the tumors is their exact duplication of the pattern of normal islets. They also resemble islet hypertrophies in their tendency to exaggerate some features of the normal islet pattern. In addition to duplicating the structure of normal and hypertrophied islets they also show the same pathological changes, such as fibrosis, hyaline degeneration, and calcification. Tumor cells, like normal islet cells, arise from duct epithelium. The sections show many examples of continuity of duct epithelium with a group of tumor cells.—W. J. A.

Hyperinsulinism and cerebral changes. Report of a case due to an islet cell adenoma of the pancreas. Malamud, N., and L. C. Grosh, Arch. Int. Med. **61**:579. 1938.

A case of chronic hypoglycemia due to an islet cell adenoma of the pancreas is reported in a married woman of 30, with clinical laboratory and pathologico-anatomic data. The clinical course of convulsions, psychotic manifestations and organic dementia is correlated with the advancing destruction of the cerebral cortex and basal ganglions. The diffuse degeneration of the brain is interpreted as a direct toxic effect of insulin on the parenchyma. It is suggested that the dextrose tolerance test in cases of hyperinsulinism is an index of the severity of the pathologico-physiologic process. This is to be understood as a change in the insulin-liver mechanism regulating dextrose metabolism.—I. B.

Changes in blood lipids during insulin treatment of schizophrenia. Randall, L. O., D. E. Cameron and J. M. Looney, Am. J. M. Sc. **195**:802. 1938.

In 5 schizophrenic patients who showed a good remission during the insulin hypoglycemic treatment, a significant rise in whole blood phospholipid, total cholesterol, and total lipid occurred. In 9 of 11 patients who did not improve during the treatment, a mild lipemia occurred during the early weeks of treatment, followed by random variations about the initial pre-medication mean. Insulin did not significantly affect the postabsorptive level of the blood lipids.—Author's Abst.

Hypoglycemia of the new-born associated with hypertrophy and hyperplasia of the islands of Langerhans. Rascoff, H., J. S. Beilly and M. Jacobi, Amer. J. Dis. Child. **55**:330. 1938.

A case of hypoglycemia apparently due to hypertrophy and hyperplasia of the islands of Langerhans in a new-born infant is reported, and the literature reviewed. Determination of blood sugar should be made for all large new-born infants who do not respond to resuscitation, or who are cyanotic and take feedings poorly. If hypoglycemia is found, and the mother is not known to be diabetic, a sugar tolerance test should be made for her. New-born infants that respond readily to inhalation of oxygen and carbon dioxide only to sink into a coma shortly afterward should be suspected of having hypoglycemia. Such infants may respond to enterally or parenterally administered dextrose. It must be used persistently. The relation of the concomitant adrenal hemorrhage present in two cases is briefly discussed.—M. B. G.

Effect of insulin on the concentration of uric acid in the blood. Rosenberg, E. F., J. Clin. Investigation **17**:233. 1938.

In 8 studies with 3 patients, administration of large doses of insulin (65 to 100 U) induced profound hypoglycemia. This was accompanied by decreasing concentration of uric acid in the whole blood (68.5%, 18.2%, 29.6%, 35.5%, 26.5%, 6.4%, 20%, and 16.1%). Control observations on these 3 patients, made when insulin was not administered, revealed in 1 instance a rise of 16% and in the other 2 a fall of 15.8% and 11.5% respectively. In 7 studies of 5 individuals, administration of large amounts of insulin, together with feeding of sufficient carbohydrate to prevent the appearance of hypoglycemic symptoms, was also associated with decreasing levels of blood uric acid, varying from 18.2% to 52%.—Author's Abst.

Protamine zinc insulin and diabetes refractory to ordinary insulin (Protamin-Zincinsulin und Insulinuempfindlicher Diabetes). Taubenhaus, M., Wien. Arch. f. inn. Med. **32**:55. 1938.

The author reports 4 cases of diabetes which were resistant to insulin, or which, because of hypertension, coronary sclerosis, hemorrhagic retinitis or some other complication, could receive no insulin at all or only small doses. Treatment with ordinary insulin had been without effect, but protamine zinc insulin produced favorable results. The fasting blood sugar, which previously had remained high, came down to nearly normal levels. This treatment does not hold good for all cases. One is cited in which it failed.—W. J. A.

Studies of the islands of Langerhans after continuous intravenous injection of dextrose. Woerner, C. A., Anat. Rec. **71**:33. 1938.

When a moderate increase in the blood sugar level is maintained by continuous intravenous injection of dextrose, a diabetic condition does not result in the guinea pig. Although the beta cells in the islands of Langerhans appear to be affected by continuous injection of sugar, in many cases there is an intense compensatory increase in the island tissue. Beta cells may arise by mitoses from preexisting beta cells, by transformation of duct and centro-acinar cells and even by direct transformation of acinar cells. With this increase in island tissue and insulin

secretion, there appears to be an increase in metabolic rate resulting in considerable loss of body weight. Hydropic degenerative changes do occur in the alpha cells and duct cells as well as the beta cells. The apparatus and methods for continuous intravenous injection into small animals are described in detail.—Author's Abst. Wistar. Bib. Service.

Experimental studies on the origin of islands of Langerhans (Experimentelle Studien zur Genese der langerhansschen Inseln des Pancreas). Wolf-Heidegger, G., Arch. f. Entwcklngsmechn. d. Organ. **135**:114. 1936.

Homotransplantations were made of the ventral and dorsal pancreatic anlagen of embryos of Bombinator pachypus and Rana esculenta in the tail bud stage. Explants were placed in the ventral body wall of the host. Some animals were kept until shortly before metamorphosis. No islands developed in the ventral pancreas transplants, but they were evident in the grafts of the dorsal pancreas 11 days after implantation. Formation of islands of Langerhans is an inherent property of pancreatic tissue.—A. T. R.

Xanthomatosis and the syndrome of diabetic exophthalmos dystosis. Wortis, S. B., A. Wolf and C. G. Dyke, Amer. J. Dis. Child. **51**:353. 1936.

Three cases of xanthomatosis in children of 2, 3, and 10 years of age are reported. Two of these children improved and were still living at the time of reporting. Treatment consisted of roentgen irradiation over areas of xanthomatosis and hypodermic injections of pitressin (to control the diabetes insipidus).—M. B. G.

PARATHYROID

Thrombocytopenic purpura hemorrhagica: Two cases treated with parathyroid hormone and calcium gluconate. Ainsworth, M. L., S. D. Edelman and R. I. Fried, J. Ped. **11**:559. 1937.

The empirical use of parathyroid hormone and calcium gluconate was followed in 3 and 4-year-old children by a prompt cessation of purpuric manifestations, and an increase in blood calcium content and in the number of platelets. The authors support Lowenberg and Ginsberg in advising the use of the hormone on the basis that it perhaps makes the blood calcium more available for the clotting cycle.—M. B. G.

Generalized osteitis-fibrosa due to retro-oesophageal parathyroid tumor. Allott, E. N., J. Jemson, Lancet **234**:609. 1938.

The author's report a pale, sallow-complexioned girl aged 19 years, unable to stand alone, with poor muscular power and bones slightly tender to palpation. She complained of pain in the chest and right arm. The right arm became numb, then loss of function occurred. The right clavicle was prominent. Laboratory examination showed no Bence-Jones protein, high serum calcium and plasma phosphatase, with low serum inorganic phosphate. X-rays showed wide-spread decalcification and cyst formation in numerous bones. The diagnosis of a parathyroid tumor was made, and the patient operated upon. Tetanic symptoms (positive Chvostek and Trousseau signs) persisted for 10 days following the operation. The calcium and phosphorus metabolism returned to normal, and the patient was completely well within a year.—L. B. S.

Variations produced in bones of growing rats by parathyroid extracts. Burrows, R. B., Am. J. Anat. **62**:237. 1938.

Parathyroid extracts, both Collip's and Hanson's, retard growth in non-parathyroidectomized young rats; they also cause atrophy of the parathyroid tissue by inhibiting cell multiplication. These effects are more readily secured after using Collip's extract. Daily administration of the extract results in a conversion of spongy bone to compact bone, especially in the legs, skull and lower jaw. Microscopic examination of the bone soon after the injections demonstrates its resorption, a decrease in number of osteoblasts, appearance of osteoclasts, and a change to fibrous tissue (osteitis fibrosa). Continuing the injection leads to a reversal of these processes; the osteoclasts disappear, osteoblasts reappear, and hypercalcification of bone occurs (marble bone disease). Further injections cause a moderate decrease in osteoblasts and a tendency for the bone to return toward normal. Similar results have been obtained on partially parathyroidectomized rats. The state of osteitis fibrosa is apparently initiated by the hormone itself or by a substance set up by it in the blood stream. Subsequent development of marble bone disease may be caused by an antibody-like substance which checks

decalcification and initiates hypercalcification. The final check on hypercalcifiation may be initiated by another antibody-like substance. These conditions differ markedly from those found in spontaneous hyperparathyroidism and may be due to impurities in the extracts rather than to direct hormonal action.—Author's Abst. Wistar Bib. Service.

THYROID

The protein content of the organs and tissues of the body after administration of thyroxine and dinitrophenol and after thyroidectomy. Addis, T., D. Karnofsky, W. Lew and L. J. Poo, J. Biol. Chem. **124:**33. 1938.

The proportion of the total body protein found in the heart, kidneys, and liver was determined in 90 control rats, in 30 rats given thyroxine and 30 given dinitrophenol. The protein content was increased by thyroxine but there was no definite change with dinitrophenol. In 41 thyroidectomized rats the proportion of total body protein in the heart and kidney was less than in the control animals.—J. M. L.

Correlation between the thyroid and the female sex organs (Zur Wechselbeziehung zwischen Schilddrüse und weiblichen Geschlechtsorganen). Alder-Mönnich, J., and R. Tiberi, Wien. Arch. f. inn. Med. **32:**41. 1938.

Guinea pigs which weighed 230-250 gm. were given daily subcutaneous injections of 0.12 to 0.25 mg. of thyroxin for 5 or 6 days. On the 7th or 8th day each animal received 2.5 R.U. of anterior pituitary prolan (Präpitan-Sanabo) subcutaneously. The animals were killed 48 hr. later. Control animals received thyroxin or prolan alone. It was found that previous treatment with thyroxin reduces the effect produced by prolan. It is stated that the place of action of this depressing influence of thyroxin is the ovary.—W. J. A.

The pituitary thyreostimulin, the thyreotrope hormone of the anterior pituitary (Ricerche sperimentali sulla tireostimulina ipofisaria, ormone tireotropo del lobo anteriore dell' ipofisi). Cattaneo, M., Arch. per le sc. med. **65:**169. 1938.

Thyrotropic hormone was injected into rabbits by the intravenous, intramuscular and intraspinal route. All animals responded with an increment in blood sugar up to 150 mg., returning to normal within 4 hours. When the animals were sacrificed one month after treatment, the thyroid showed the usual histological changes as vascularization, hyperplasia of the parenchyma, change of the epithelial cells from the vascular to the cylindric type with hypertrophic Golgi apparatus and nuclei in mitosis. The colloid showed numerous vacuoli and was pale. The thyrotropic extract does not cause hyperglucemia during somniphen narcosis, which itself does not influence the blood sugar. The response to insulin during this narcosis is greatly enhanced. Thyroidectomy abolished the hyperglucemic reaction whereas it increased the response to insulin. The theory is offered that the hormone acts via the nervous system.—A. E. M.

Endocrine affections of the skeleton. The thyroid (Les affections endocriniennes du squelette. La thyroide). Coryn, G., Presse méd. **46:**228. 1938.

Cartilage changes in thyroidectomized rabbits and in animals treated with excessive doses of thyroid are reported. Some cases of infantile and adult hypothyroidism and responses to thyroxine treatment are described. One case of progressive hyperthyroidism of 15 years' duration is reported. Calcium and phosphorus in the blood were normal but the phosphatase was increased. The urinary calcium elimination was considerably augmented. Calcium balance could be obtained by daily ingestion of 0.65 gm. Ca. The skeleton was decalcified, and spontaneous fractures occurred frequently. Partial thyroidectomy with removal of an adenoma brought about perfect cure. The parathyroids were normal.—A. E. M.

Exophthalmic goiter in a boy two and one-half years of age: Review of the literature. Crile, G. Jr., and J. L. Blanton, Amer. J. Dis. Child. **53:**1039. 1937.

The authors find that only 7 cases of clear-cut hyperthyroidism in children under 5 years of age have been reported in the American literature, only one of them being in a boy. They report a case of a boy of 2½ years, who showed the following symptoms of hyperthyroidism: persistent tachycardia, loss of weight, tremors, exophthalmos, goiter, marked muscular weakness and nervousness. The blood iodine was 14.9 γ %, blood cholesterol, 136 mg. %, blood sugar (139 and 182 mg.). Ossification and dentition were advanced. A subtotal thyroidectomy was successfully performed. The gland was about 5 times the normal size and extremely

vascular. The pathological diagnosis was hyperplasia and moderately involuting goiter. No adenomas were present. The child made a good recovery.—M. B. G.

Exophthalmos in Grave's disease. Daniels, L. P., Acta med. Scandinav. **95**:539. 1938.

This is a dissertation on the mechanism for the production of exophthalmos. The author explained condition as due to the action of thyrotropic hormone of pituitary as a result of stimulation of centers in the diencephalon.—J. M. L.

The effect of experimental hyperthyroidism on the vitamin B_1 content of some rat tissues. Drill, V. A., Am. J. Physiol. **122**:486. 1938.

The feeding of 100 mg. of desiccated thyroid per rat per day for 17 days produced losses in weight and, in some cases, death. Compared with controls receiving the same quantity of synthetic diet daily, these animals had about the same amount of vitamin B_1 in the spleen, a reduced amount in the kidney and heart, and less than half as much in the liver. Injecting 500 γ of vitamin B_1 daily into a second series of controls and thyroid-fed rats resulted in similar differences due to hyperthyroidism, except that the content of the heart was raised. Deaths and losses in weight of hyperthyroid rats were not due to lack of vitamin B_1 as such. Thyroid feeding did not affect the urinary excretion of the vitamin.—H. W. Schultz.

Effect of prolonged administration of thyroxin upon adrenals and blood preosure in the rabbit (Über den Einfluss einer langwährenden Thyroxinverabreichung auf die Nebennieren und auf den Blutdruck der Kaninchen). Gerlei, F., Endokrinologie **19**:387. 1938.

Thirty-two rabbits were given thyroxin in daily subcutaneous doses ranging from ¼ to 4 mg. over a period of 2 years, in order to determine whether such prolonged thyroxin poisoning would be followed by a cirrhosis of the liver. In 6 of the 32 animals there were found, in the center of the liver lobules, small fibrous masses which apparently had followed an earlier induced central necrosis of the lobule. The adrenal glands were hypertrophied, on the average 50%. The hypertrophy involved principally the cortex; the enlargement of the medulla was only moderate. As evidence that the hypertrophied adrenals were exhibiting increased functional activity it was found that the blood pressure was higher in the thyroxin-injected animals than in the controls.—W. J. A.

On the occurrence of pharyngeal infections in exopthalmic goiter. King, J. T. Bull. Johns Hopkins Hosp. **62**:593. 1938.

The high incidence of pharyngeal infections as a prelude to the development of exophthalmic goiter suggests a possible etiological relationship.—A. G.

The effect of hyperthyroidism upon the metabolism of vitamin C. Lewis, R. A. Bull. Johns Hopkins Hosp. **63**:31. 1938.

The vitamin-C excretion of 5 hyperthyroid patients was subnormal but reached normal values in 4 of them, following thyroidectomy.—A. G.

Regarding muscle changes in Basedow's disease (Zur Frage der Muskelveränderungen bei Morbus Basedowii). Liechti, H., Endokrinologie **20**:81. 1938.

Upon histological study of pieces of striated muscle from 15 cases of Basedow's disease the author was unable to observe the thickening of capillary wall and the pericapillary edema as described by Eppinger. He concludes that the metabolic disturbance in the muscles cannot be attributed to serous inflammation.—W. J. A.

Direct measurement of height of thyroid epithelium. A method of assay of thyrotropic substance; clinical application. Rawson, R. W., and P. Starr, Arch. Int. Med. **61**:726. 1938.

The increased height of the acinar epithelium of the guinea-pig thyroid induced by thyrotropic substance may be found by direct micrometer measurements, and the hyperplasia throughout the gland may be represented by a frequency curve derived from these measurements. Increasing doses of a solution containing thyrotropic substance produce increasing shifts of such curves to the right. The extract of 50 cc. of urine of normal persons produces a slight shift to the right. An extract of 50 cc. of urine of nonmyxedematous persons with a low basal metabolic rate may or may not produce a greater effect. The extract of 50 cc. of urine of men totally thyroidectomized for heart disease produced a still greater effect. The extract of 50

cc. of urine of men and women with hyperthyroidism, with or without exophthalmos, produced no effect except in 2 cases.—I. B.

Cretinoid epiphyseal dysgenesis. Reilly, W. A., and F. S. Smyth, J. Ped. **11**:786. 1937.

Peculiar stippling of the epiphyses in endemic cretinism has been noted by several observers. The authors describe a similar condition in several children who had severe hypothyroidism, which was characterized by the formation of epiphyses from multiple loci of ossification, slow calcification, a bilateral distribution and improvement under thyroid therapy. Dysgenesis may be confused with the chondrodystrophies described by Legg, Perthes and others.—M. B. G.

Association of hyperthyroidism with pulmonary tuberculosis. A review of the literature and report of 23 cases. Rose, E., and H. U. Hopkins. Arch. Int. Med. **61**:631. 1938.

This is a general discussion of the subject with a brief review of the progress of 23 patients with coexistent hyperthyroidism and pulmonary tuberculosis. The authors find that the administration of iodine to thyrotoxic patients with pulmonary tuberculosis does not tend to precipitate any immediate exacerbation or extension of the tuberculosis. An operation on the thyroid gland is usually tolerated by patients with pulmonary tuberculosis. The coexistence of pulmonary tuberculosis does not impair materially the prospect for the successful treatment (irradiation or operation) of hyperthyroidism. The relief of hyperthyroidism does not seem to influence favorably the prognosis for patients with associated active pulmonary tuberculosis. The clinical picture of hyperthyroidism, without characteristic structural changes in the thyroid gland, may occur in patients with active pulmonary tuberculosis.—I. B.

The creatine tolerance test in the differential diagnosis of Graves' disease and allied conditions. Sohval, A. R., F. H. King and Miriam Reiner, Am. J. M. Sc. **195**:608. 1938.

The Shorr creatine-tolerance test was made on 75 patients 15 of whom had Graves's disease and the remainder a variety of border-line conditions such as essential hypertension and menopause. The test gave positive results in 90% of the Graves's cases but also in 18% of control cases. The test is regarded as of only moderate value in the differential diagnosis of Graves's disease.—R. G. H.

Medical treatment of hyperthyroidism with a high fat diet. Soskin, S., and I. A. Mirsky, J.A.M.A. **110**:1337. 1938.

The authors cite a case of acute and severe hyperthyroidism in which the patient refused to submit to thyroidectomy. The treatment consisted of a high fat diet: 90 gm. protein, 230 gm. fat and 90 gm. carbohydrates, making total calories 2,790. For 23 days 3 gm. daily of cholesterol was added to the diet. Rest in bed was enforced. No other form of therapy was employed. The patient made a complete recovery, which has persisted for almost 2 years.—R. G. H.

Comparison of tissue metabolism in normal, spayed, spayed-thyroidectomized and hypophysectomized female rats. Victor, J., and Dorothy H. Andersen, Am. J. Physiol. **122**: 296. 1938.

Metabolic studies were made on isolated liver and kidney tissues from normal and spayed rats (98-110 days old), spayed-thyroidectomized (120-130 days) and hypophysectomized rats (105-125 days). Spaying, or spaying and thyroidectomy compared with normal tissue lowers the respiratory rate of liver about 19% and lowers the R.Q. of kidney; hypophysectomy shows the same effects, but in addition lowers the R.Q. of the liver, the respiratory rate of kidney and further lowers the R.Q. of the kidney. Thyroidectomy after spaying shows no significant difference from spaying alone; hypophysectomy, however, lowers the respiratory rates and quotients of both liver and kidney from the spayed level. Hypophysectomized, as compared with spayed-thyroidectomized rats, shows significant decreases in liver R.Q. and lower renal respiratory rates and quotients. Observations on respiratory rates of tissues of hypophysectomized rats confirm the observations on total basal metabolism, but not the changes in the basal R.Q. in intact animals.—S. R. Tipton.